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KINETICS OF HYDROGEN ASSIMILATION IN METHANE FERMENTATION

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KINETICS OF HYDROGEN ASSIMILATION IN METHANE FERMENTATION

by

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Sanitary Engineering Research Laboratory
College of Engineering
and
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University of California
Berkeley

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TABLE OF CONTENTS

Page

LIST OF TABLES vi

LIST OF FIGURES viii

Chapter

I. INTRODUCTION 1

Objectives of Investigation 3
Acknowledgments 3

II. THEORY 4

The Methane Fermentation from Hydrogen 4

General . . . .
Methane Fermentation from Hydrogen and Carbon Dioxide 4
Other Compounds Formed from Hydrogen and Carbon Dioxide 9

Hydrogen Assimilating Methane Bacteria 10

Kinetic Characteristics 10
Growth Rates 10
Cell Yields 11
Substrate Utilization Rates 11

Physiological Requirements 12

Role of Hydrogen 13

Occurrence of Hydrogen and Formate 13
Significance of Hydrogen and Formate 17

Continuous Culture Theory 19

Continuous Flow Fermentation 19
Kinetic Description 19
Cell Separation and Recycle 24

Chapter Page

III. EXPERIMENTAL PROCEDURES 26

Hydrogen Enrichment Kinetic Study 26

- Bacterial Culture 26
- Experimental Apparatus 27
- Substrate Characteristics 33
  - Substrate Gas 33
  - Liquid Substrate 34
- Analytical Methods 37
  - Suspended Solids 37
  - Gas Analyses 39
- Operation 39
  - Startup 39
  - Material Balances 40
  - Sampling and Analyses 41
- COD Balance Calculations 41
  - Gas Composition 42
  - Gas Balance 42
  - Diffusion Losses 44
  - Conversion Factors 46
- Kinetic Evaluation 46
  - Approach 46
  - Calculations 48

Hydrogen Supplementation 51

- Experimental Apparatus 51
- Substrate Characteristics 55
  - Substrate Gas 55
  - Liquid Substrate 55
- Analytical Methods 55
- Operation 55
- Calculations 58

IV. HYDROGEN ENRICHMENT KINETIC STUDY 59

Results 59

- Determination of Kinetic Constants 61
- Statistical Evaluation of Results 68
  - COD Material Balance 70
  - Similarity of Enrichment Systems 73
  - Volatile Acids 73

Chapter Page

Discussion 74

  Kinetic Evaluation of Enrichment Cultures 74
    Generation Time 75
    Substrate Removal Rate Coefficients 77
    Saturation Constant 80
    Yield Coefficient 82
    Organism Decay Term 83
    Methane Production Rates 86
  Summary 88

Hydrogen Supplemented Fermentations 89

  Results 89
    Culture Homogeneity 94
    Hydrogen Supplementation 94
  Discussion 96

Conclusions 99

GLOSSARY 103

APPENDICES

A. Characteristics of the Substrate 107

B. Determination of Fermentation Gases by Gas Chromatography 109

C. Method for Quantitative Containment and Recovery of Fermentation Gases 116

D. Diffusion Correction in Hydrogen Enrichment Study . . . 120

E. Hydrogen Enrichment Growth and Kinetic Study Data . . . 123

F. Hydrogen Supplementation Study Data 130

REFERENCES 135

LIST OF TABLES

Table Page

I. Substrate Specificity of the Methane Bacteria 5

II. Products of Escherichia coli Fermentations 15

III. Liquid Substrate Composition for Enrichment Culture Fermentations 36

IV. Liquid Substrate Characteristics for Enrichment Culture Fermentations 36

V. Gas COD Conversion Factors 46

VI. Liquid Substrate Composition for Hydrogen Supplementation Study 56

VII. Liquid Substrate Characteristics for Hydrogen Supplementation Study 56

VIII. Growth and Kinetic Data--Enrichment Study 62

IX. Growth and Kinetic Characteristics--Enrichment Study . . 62

X. Enrichment Growth and Kinetic Constants 67

XI. Maximum Measured Gas Transfer Rates 68

XII. Statistical Analysis of COD Balance Indices 71

XIII. Variation of COD Balance Indices with Cell Residence Time 72

XIV. Generation Times and Yield Coefficients of Methane Bacteria 76

XV. Typical Biological Process Constants 79

XVI. Maximum Methane COD Production Rates 87

XVII. System 3 Growth and Kinetic Data 90

XVIII. System 4 Growth and Kinetic Data 91

Table Page

XIX. Deleted . . . . --

XX. Equipment and Program of Operation 110

XXI. Relative Thermal Conductivity Factors 111

XXII. Analytical Precision with Standard Gas Mixtures 115

XXIII. Gas Container Program of Operation 118

XXIV. General Data--Enrichment Study 124

XXV. System 1 Gas Flow Rates and Analyses 125

XXVI. System 2 Gas Flow Rates and Analyses 126

XXVII. System 1 Adjusted Gas Flow Rates 127

XXVIII. System 2 Adjusted Gas Flow Rates 128

XXIX. COD Balance--Enrichment Study 129

XXX. System 3 General Data 131

XXXI. System 4 General Data 133

LIST OF FIGURES

Figure Page

1. Carbon Pathways in Methane Fermentation 8

2. Products of the Escherichia coli Fermentation of Glucose 14

3. Single Stage Continuous Flow Reactor System with Cell Separation and Cell Recycle 20

4. Determination of Y and k_d--Cell Continuity Equation . . . 23

5. Determination of $\hat{q}$ and K_s--Michaelis-Menten Equation . . 23

6. Enrichment Study Continuous Culture Apparatus--System 1 and 2 28

7. Gas Feed System and Program Timer Panel 30

8. Enrichment Study Reactor. Systems 1 and 2 31

9. Composition of Substrate Gas Mixture with Variation of Cell Residence Time 35

10. Analysis of Reactor Gases and Contents--Enrichment Study 38

11. Hydrogen Supplementation Study Reactors Systems 3 and 4 . 52

12. Gas and Liquid Recirculation Flow Sheet for Hydrogen Supplementation Study--System 3 and 4 53

13. Determination of Yield Constant and Specific Organism Decay Rate--Hydrogen COD Removal Basis 63

14. Determination of Yield Constant and Specific Organism Decay Rate--Total COD Removal Basis 64

15. Determination of Saturation Constant (K_s) and Maximum Substrate Metabolic Coefficient ($\hat{q}_{H_2}$)--Hydrogen COD Removal Basis 65

16. Determination of Saturation Constant (K_s) and Maximum Substrate Metabolic Coefficient ($\hat{q}_T$)--Total COD Removal Basis 66

Figure Page

17. Comparison of Steady State Data with the Michaelis-Menten Equation of Best Fit--Hydrogen COD Removal Basis 69

18. Hydrogen Supplementation Study Characteristics 92

19. Hydrogen Supplementation Study General Characteristics . 93

20. Column Arrangement in Chromatograph 112

21. Typical Gas Analysis Chromatogram 114

22. Container and Appurtenances 117

I. INTRODUCTION

The anaerobic fermentation of organic wastes is a stepwise degradation process which is initiated with solubilization of particulate matter and the production of volatile acids from simple and complex organic molecules, and is completed by the evolution of methane and carbon dioxide. The methane-producing stage appears to be the rate-limiting step in the overall process. Methane bacteria appear to grow at slower rates than do most other anaerobic organisms. A mixed population of methane bacteria is often required to ferment completely a single compound.

The volatile fatty acids and carbon dioxide are two carbon precursors of methane known to be significant in anaerobic fermentation. Methane is derived (1) by decarboxylation of acetic acid and (2) by carbon dioxide reduction accompanying the oxidation of volatile acids and of molecular hydrogen. Digester failure is accompanied normally by a reduction of pH arising in part from accumulation of volatile acids and by a decrease in methane production. Research in the anaerobic biological waste treatment field has been directed generally toward metabolism of the volatile fatty acids, particularly of acetic and propionic acids. This emphasis has arisen in part because of the association of increasing acetic and propionic acid concentrations with failing fermentations.

In contrast, the subject of methane fermentation from hydrogen has received relatively little attention. Even though hydrogen is a decomposition product in the glucose fermentation by E. coli, only negligible quantities of hydrogen normally are found in the product gases of an active methane fermentation. Hydrogen can be combined biologically with carbon dioxide to form methane or reduced compounds other than methane, or it may be combined with organic compounds by hydrogenation reactions. Because hydrogen normally does not accumulate as a fermentation product, it must be an intermediate in the methane fermentation which is assimilated rapidly upon formation.

Data are not available to permit assessment of either the magnitude of hydrogen assimilation in anaerobic fermentation processes, or the ultimate distribution of the assimilated hydrogen in the final products of the fermentation. Particularly lacking is information on the kinetics and magnitude of methane production from hydrogen. Such data are needed to define the contribution of the hydrogen assimilating methane bacteria to the overall methane production.

Hydrogen in its intermediate role also may be implicated in the methane fermentation of volatile acids. If hydrogen is the rate limiting component in one of the complex series of reactions by which methane is derived from volatile acids, the addition of hydrogen to the fermentation may increase the methane productivity of the fermentation. The effect would be advantageous not only from the viewpoint of improved digestion of organic matter, but also in the recovery of the additive as a methane byproduct.

Objectives of Investigation

The specific objectives of this investigation were as follows:

1. To determine the microbiological kinetic constants of the methane bacteria which ferment hydrogen, utilizing continuous-flow fermentors.
2. To evaluate experimentally the effect of hydrogen on the methane fermentation of volatile acids.

Acknowledgments

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The counsel of Dr. C. R. Wilke, Professor of Chemical Engineering at the University of California at Berkeley, is gratefully acknowledged. Thanks are also due to Jan Scherfig for review of the manuscript and to C. F. Reid and C. H. Huang for assistance in performance of the many analyses required for this investigation.

II. THEORY

The Methane Fermentation from Hydrogen

General

The methane producing bacteria are a distinct physiological group of organisms, the individual species of which exhibit strict substrate specificity. Table I, compiled from Barker [1], summarizes the substrate specificity of the known methane-producing bacteria, and indicates that pure cultures cannot convert completely some of the compounds to methane and carbon dioxide. A mixed population may be required for the complete conversion of the long-chain volatile fatty acids such as butyric, valeric, and caproic acids.

Methane bacteria are difficult to isolate and maintain in pure culture. Limited information on the biological and physiological characteristics of these bacteria is available.

Methane Fermentation from Carbon Dioxide and Hydrogen

The stoichiometry of carbon dioxide reduction to methane by hydrogen oxidation was established by Sohngen [2]:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (1)$$

The specific reaction pathways through which carbon dioxide is reduced to methane are unknown. Stephenson and Stickland [3] reported isolation of a bacterium in pure culture which was capable

TABLE I

SUBSTRATE SPECIFICITY OF THE METHANE BACTERIA[a]

Bacterium	Compounds Oxidized
Methanobacterium formicicum	H_2, CO, formate
Methanococcus vannielii	H_2, formate
Methanosarcina barkerii	H_2, CO, methanol, acetate
Methanobacillus omelianskii	H_2, ethanol, primary and secondary alcohols
Methanobacterium sohngenii	Acetate, butyrate
Methanococcus mazei	Acetate, butyrate
Methanosarcina methanica	Acetate, butyrate
Methanobacterium propionicum	Propionate
Methanobacterium suboxydans	Butyrate, valerate, caproate

[a] After Barker [1]

of reducing carbon dioxide, carbon monoxide, formate, formaldehyde, and methanol to methane with the oxidation of hydrogen. The validity of these results has been questioned by Stadtman and Barker [4], because Stephenson and Stickland presented insufficient evidence of the purity of their culture. Results from the pure culture experiments of Barker [5], Stadtman and Barker [4] and Pine [6] have indicated that formate is not a normal intermediate in the reduction of carbon dioxide to methane. Carroll and Hungate [7] observed that C^{14} labelled formate was converted quantitatively to C^{14} carbon dioxide and hydrogen in bovine rumen contents. They also noted that the maximum potential rate of conversion of hydrogen and carbon dioxide to methane was greater than the maximum rate of decomposition of formate in rumen contents, with the result that hydrogen did not accumulate in significant concentration.

Kluyver and Schnellen [8] have studied the fermentation of one carbon compounds with three species of methane bacteria. They established that M. omelianskii was unable to reduce one carbon compounds at a significant rate except for carbon dioxide, which was reduced rapidly. M. barkerii could not reduce formate or formaldehyde, but fermented methanol, acetate, and carbon monoxide. M. formicicum was found to be able to utilize both carbon monoxide and formate, with carbon dioxide as the intermediate. Such evidence appears to preclude one carbon compounds other than carbon dioxide as intermediates in methane formation.

Stadtman and Barker [9] concluded that a Methanosarcina species, which was able to ferment acetate and methanol, could also reduce carbon dioxide to methane in the presence of hydrogen. It appeared that one methane organism utilized three distinct mechanisms for methane formation, one for each of the three substrates. Barker [1] hypothesized that a common methylated intermediate was the vehicle of methane formation from all three individual sources. The scheme developed by Barker is presented in Figure 1.

In Figure 1, compound XH is an unidentified organic compound or series of compounds which combine with carbon dioxide. After successive reductive steps, a common methylated intermediate XCH_3 is produced. Acetate and methanol also react with XH to give the methyl intermediate, the reduction of which yields methane and regenerates the acceptor XH.

Recent observations have provided some experimental support for the pathway of methane formation proposed by Barker. Wood and Wolfe [10]report that tetrahydrofolate derivatives are significant in methane formation. Tetrahydrofolic acid appears to be involved in the transfer of one carbon groups such as formyl and hydroxymethyl groups, according to Thimann [11]. Brilly et al. [12] linked the oxidation of formate to carbon dioxide with reduction of ferridoxin, which is an iron-containing protein of low electrode potential. Apparently, reduced ferridoxin transferred its hydrogen to the coenzyme NAD as follows:

$$HCOOH + (Fd)_{OX} \rightarrow CO_2 + (Fd)_{RD} \qquad (2)$$

$$(Fd)_{RD} + NAD \rightarrow (Fd)_{OX} + NADH + H^+ \qquad (3)$$

Under certain conditions, Wolin et al. [13] have shown that ferridoxin stimulates methane production in the presence of pyruvate,

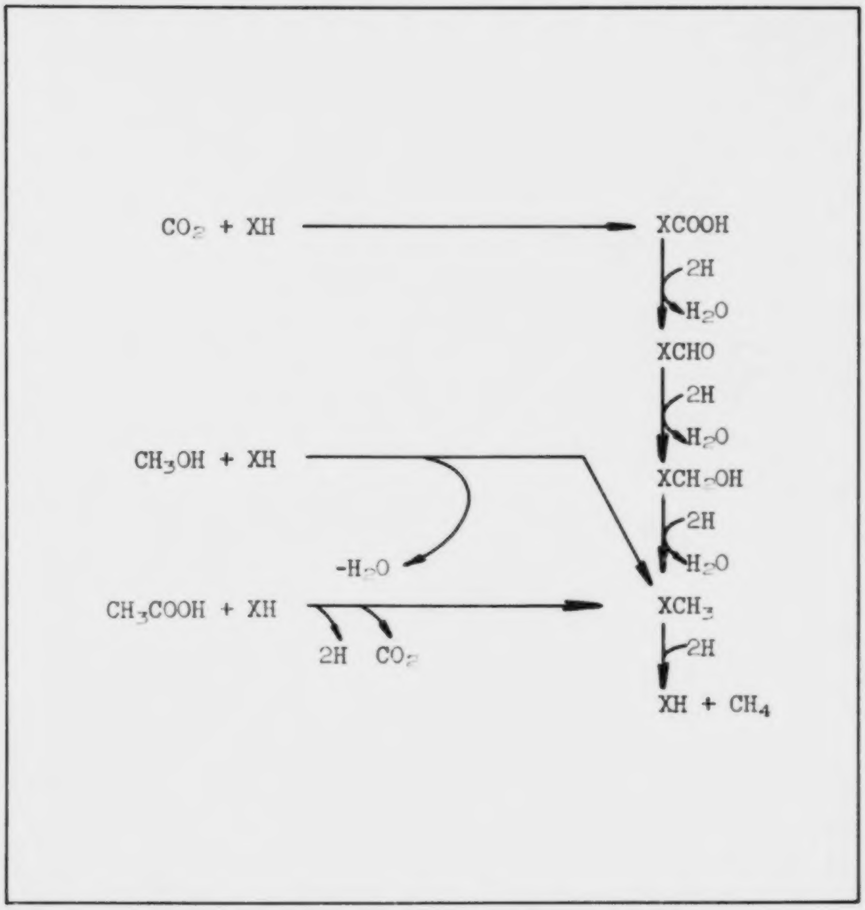

CO2 + XH
XCOOH
2H
H2O
XCHO
2H
H2O
CH3OH + XH
XCH2OH
2H
H2O
-H2O
CH3COOH + XH
XCH3
2H CO2
2H
XH + CH4

FIGURE 1. CARBON PATHWAYS IN METHANE FORMATION (AFTER BARKER [1])

hydrogen and carbon dioxide. Ferridoxin also has been detected in M. omelianskii [14].

Blaylock and Stadtman [15,16] have demonstrated that disrupted cell preparations of M. barkerii produced methane from methylcobalamin with pyruvate as the electron acceptor. Both the soluble and particulate fractions of the cell were required. Intact cells did not form methane from methylcobalamin. M. barkerii contains sufficient cobamide derivatives to suggest that these compounds may be important in methane formation [15].

Other Compounds Formed From Hydrogen and Carbon Dioxide

Several reactions have been studied in which carbon dioxide is not completely reduced to methane by hydrogen. Wieringa [17] has isolated an organism, Clostridium aceticum, which was capable of reducing carbon dioxide to acetic acid as follows:

$$2\ CO_2 + 4H_2 \rightarrow CH_3CHOOH + 2H_2O \qquad (4)$$

Barker et al. [18] have studied the fermentation of uric acid to acetic acid by Clostridium acidi-urici. This is one of a class of reactions whereby hydrogen can react with an organic electron acceptor to form acetic acid (methane carbon precursor). The general reaction is described as follows:

$$2\ CO_2 + 4\ H_2A \rightarrow 4A + CH_3COOH + H_2O \qquad (5)$$

where H_2A is the organic electron acceptor.

According to Pine and Barker [19], Butyribacterium rettgeri incorporates carbon dioxide into both carbons of acetate and exchanges the acetate carboxyl carbon with carbon dioxide. Wood [20] has demonstrated that Clostridium thermoaceticum possessed a similar mechanism. Barker and Elsden [21] have studied uric acid fermentation by Clostridium cylindrosporum. When this organism ferments uric acid in the presence of C^{14}-carbon dioxide, the isotope is recovered both in the methyl group of acetate and the carboxyl group of glycine.

Because of reactions such as these, the importance of which in anaerobic fermentation is unknown, hydrogen may not be recoverable completely as methane or in cell synthesis in an anaerobic fermentation. It appears that the remaining hydrogen may combine biologically with complex organic substances to form simpler compounds which can be readily fermented to methane.

Hydrogen Assimilating Methane Bacteria

Kinetic Characteristics

Growth Rates.--Quantitative data describing the growth kinetics of hydrogen assimilating methane bacteria are unavailable presently. Crude estimates of the generation times of methane bacteria may be obtained from pure culture batch studies. Mylroie and Hungate [22], working with M. formicicum at 38°C in an atmosphere of hydrogen and carbon dioxide, observed the first appearance of colonies in about three days. Stadtman and Barker [4] obtained a vigorous fermentation of formate with M. vannielii in 16 to 36 hours. Formate was decomposed to carbon dioxide and hydrogen, whereupon methane was produced by reduction of carbon dioxide to methane. In contrast, Stadtman and Barker [23] observed that 25 to 60 days were required for the start of gas production from propionic acid with M. propionicum at 30°C.

Assessment of the above results should consider that batch cultures pass through a lag phase of unknown duration before logarithmic growth occurs.

Cell Yields.--Mylroie and Hungate [22] have obtained quantitative data for the yield of *M. formicicum* cells grown in batch culture on a mixture of 80 percent hydrogen and 20 percent carbon dioxide. After 24 days of incubation, a cell yield of 0.417 mg dry weight cells/millimole hydrogen was measured. This is equivalent to 0.0261 mg dry weight of cells/mg hydrogen chemical oxygen demand (H_2-COD). The foregoing represents the only available information on the kinetics of hydrogen assimilating bacteria.

Substrate Utilization Rates.--Substrate removal rates based on a hydrogen energy source have not been determined, although Barker [24] reported the results of an experiment with *M. omelianskii* which permits an estimate to be made. Barker measured the influence of carbon dioxide concentration on the rate of carbon dioxide reduction with hydrogen. Data were developed with a single culture vessel in which the accumulation of product methane did not appear to retard the reaction. The maximum hydrogen removal rate can be calculated from the data of Barker as 6100 cu cm H_2/mg cell N-hr. This rate is equivalent to 12 mg H_2-COD/mg VSS-day if it is assumed that bacterial cells are 90 percent volatile (organic) matter and about 10 percent nitrogen. No other data are available for comparison.

In the same study Barker determined a saturation constant for carbon dioxide of 7×10^{-5}M. At equilibrium conditions and 38°C, this saturation constant is equivalent to an atmosphere of 0.28

percent carbon dioxide. Assuming this constant is applicable to the hydrogen assimilating bacteria in sewage sludge fermentation, it is unlikely that carbon dioxide (normally 20-30 percent in digester gases), would limit the rate of hydrogen uptake. This may explain why increasing the partial pressure of carbon dioxide in sewage sludge digesters has not been very successful in improving methane production.

Physiological Requirements

Barker [1] enumerated the nutritional requirements for the species studied in pure culture as the usual nutritive salts, carbon dioxide, a reducing agent, a single oxidizable compound suitable for the organism, and an ammonium salt as a source of nitrogen. The addition of extracts containing amino acids, growth factors, and other nutritional supplements to synthetic media did not appear to have a beneficial effect on the rate of growth of the few species studied. Mylroie and Hungate [22] observed that yeast extract (growth factors) did not decrease the time required for appearance of colonies of *M. formicicum* However, in the present work, the addition of yeast extract to the culture media was required to obtain hydrogen uptake in enrichment culture. Because growth factors have not been required for the pure culture of methane bacteria, the yeast extract may have been required for growth of the contaminating organism which created a suitable environment for the growth of hydrogen assimilating methane bacteria.

According to Barker [5] the optimal pH range for growth of *M. omelianskii* with hydrogen and carbon dioxide is 6.0-7.8. Mylroie and Hungate [22] have reported a pH range of 6.6-7.8 with M. formicicum. The upper limit apparently results from the absence of dissolved carbon dioxide at pH 7.8. Barker [24] has suggested that dissolved carbon dioxide is in the form of carbon dioxide (rather than bicarbonate) which is assimilated by methane bacteria.

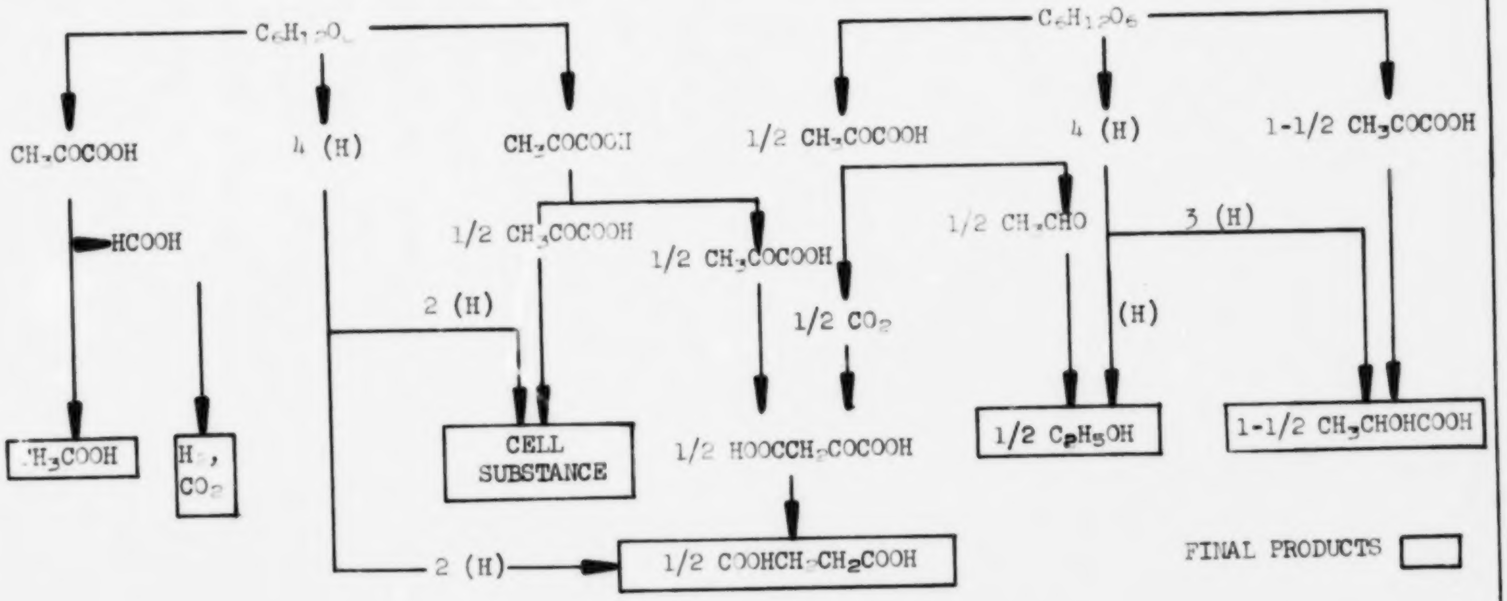
Role of Hydrogen

Occurrence of Hydrogen and Formate

Hydrogen is a byproduct of the glycolytic fermentation pathway, and in the absence of assimilation would be an expected product of anaerobic fermentations. A general scheme for the E. coli fermentation of glucose is illustrated in Figure 2, taken from Thimann [11]. The quantities of hydrogen and other compounds produced depend on the relative activities of the enzymes taking part in the fermentation, and apparently vary with the growth conditions.

The effect of pH on the products of glucose fermentation is shown in Table II. When the pH is maintained at 6.2, E. coli produced little formic acid but substantial hydrogen and carbon dioxide. In contrast, primarily formic acid is formed at pH 7.8 with limited production of hydrogen and carbon dioxide. The enzyme hydrogenlyase, which exhibits optimum activity at pH 6.0 appears to have little activity at pH 7.8. The hydrogen utilizing methane bacteria, with the exception of M. omelianskii and M. barkerii, appear to be capable of formate uptake [1].

In a properly functioning fermentation of sewage sludge, hydrogen and formic acid are present in minimal concentrations. Burgess and Wood [26], collating data from 48 independent fermentation


C6H12O6
CH3COCOOH
4 (H)
CH3COCOOH
HCOOH
CH3COOH
H2, CO2
1/2 CH3COCOOH
2 (H)
CELL SUBSTANCE
2 (H)
1/2 CH3COCOOH
1/2 HOOCCH2COCOOH
1/2 COOHCH2CH2COOH
C6H12O6
1/2 CH3COCOOH
4 (H)
1-1/2 CH3COCOOH
1/2 CO2
1/2 CH3CHO
3 (H)
(H)
1/2 C2H5OH
1-1/2 CH3CHOHCOOH
FINAL PRODUCTS

$$\underset{\text{(GLUCOSE)}}{2\ C_6H_{12}O_6} = \underset{\text{(LACTATE)}}{1\text{-}1/2\ CH_3CHOHCOOH} + \underset{\text{(ACETATE)}}{CH_3COOH} + \underset{\text{(SUCCINATE)}}{1/2\ HOOCCH_2CH_2COOH} + H_2 + CO_2 + \underset{\text{(ETHANOL)}}{1/2\ C_2H_5OH} + \text{CELL SUBSTANCE}$$

FIGURE 2. PRODUCTS OF THE ESCHERICHIA COLI FERMENTATION OF GLUCOSE (AFTER THIMANN [11])

TABLE II

PRODUCTS OF *ESCHERICHIA COLI* FERMENTATION

Products	m Mole per 100 Moles Glucose Fermented	
	pH 6.2	pH 7.8
2-3 Butanediol	0.3	0.26
Acetoin	0.007	0.19
Glycerol	1.42	0.32
Ethanol	49.8	50.5
Formic Acid	2.43	86.0
Acetic Acid	36.5	38.7
Lactic Acid	79.5	70.0
Succinic Acid	10.7	14.8
Carbon Dioxide	88.0	1.75
Hydrogen	75.0	0.26
Carbon Recovered (%)	91.2	94.7

Data from Wood [25].

gas analyses, reported an average hydrogen content of less than three percent in the thirteen cases where hydrogen was detected. Imhoff [27] has suggested that sludge gas could be freed of hydrogen economically by recirculating the gas mixture through actively digesting sludge. Buswell [28] reported that the presence of hydrogen indicates a lack of process completion and the onset of poor digestion, particularly with carbohydrate fermentation. Many of the hydrogen analyses have been performed with methods whose precision often equalled the quantities reported; consequently, the data of many studies has questionable value. The use of gas chromatographic methods of analysis such as employed in this investigation should improve the accuracy and precision of such data.

Formic acid concentrations and assimilation rates have been reported in recent years by Leete [29] and others. Leete [29] observed steady state formic acid concentrations of 48 and 57 mg/l (as acetic acid) in two active laboratory sludge digestions. Pohland and Bloodgood [30] studied failing digestion at several temperatures. At 97°F, the maximum formic acid concentration was observed to be 198 mg/l when the total volatile acids were about 3800 mg/l. The average value was 50 mg/l. Formic acid never exceeded 6 percent of the total volatile acids. Keefer and Urtes [31,32] studied rates of volatile acid utilization with digesting sludge. Their reactors were batch operated at 37°C. They observed that formic acid was assimilated more rapidly than either acetic, propionic, or butyric acids.

Significance of Hydrogen and Formate

Because hydrogen and formic acid are byproducts of the glycolytic pathway of anaerobic metabolism, the low concentrations of hydrogen and formic acid frequently observed in active methane fermentations indicate that each is rapidly assimilated upon formation. Rapid utilization of these compounds requires the presence of active methane-producing or other bacteria capable of hydrogen and formic acid uptake. Indeed Smith [33], who studied the production of methane in serial dilutions of sludge with several substrates, has observed that the hydrogen assimilating methane bacteria were more numerous in sludge than methane bacteria which utilize acetate.

Jeris and McCarty [34] reported that acetate is a precursor of about 70 percent of the methane produced in a mixed culture fermentation. Smith and Mah [35] reported similar results with acetate. Smith [36] presented additional evidence to show that propionic and butyric acids may account for another 13 and 8 percent respectively of the methane produced. It follows that by difference the methane derived from carbon dioxide reduction with hydrogen is likely to be less than 10 percent of the total methane produced.

Smith and Mah [35] considered the energetics of methane formation from acetate and hydrogen and reported that the free energy yield for the conversion of acetate to methane is about one-third of that derived from carbon dioxide reduction with hydrogen. It was suggested that hydrogen may be a significant source of energy for growth of methane-producing bacteria in sludge, even though it accounted only for a small fraction of the methane produced.

Scherfig [37] has investigated the energy relationships of methane formation from acetate and carbon dioxide. Calculations based upon free energies indicate that chemical equilibria in normal digestion may preclude hydrogen availability for carbon dioxide reduction. The same calculations also show that the concentration of formic acid at pH 7 will be less than 6.0 mg/l (acetic acid). The results suggest that the presence of glycolytic products, such as found in digesting organic material, can regulate the rate of energy production from carbon dioxide reduction.

It appears that hydrogen and acetate metabolism by methane bacteria may be related energetically and may have common enzyme or coenzyme requirements. Barker [1] has suggested that a common intermediate lies on the metabolic pathway from acetate and carbon dioxide to methane. He also indicated that the direct combination of acetate and methanol with a common acceptor XH (Figure 1) may inhibit the utilization of carbon dioxide by decreasing the availability of uncomplexed acceptor. If the reverse is possible, then an excess of hydrogen may also exert an inhibitory effect on the decomposition of acetate. The report of Buswell [28] that hydrogen production has indicated the onset of digester failure suggests the possibility of "hydrogen inhibition."

It is obvious that information on the role of hydrogen and formate in digestion is in conflict. Hydrogen appears to be converted rapidly to methane or to react with complex organic materials to form simpler compounds. One of these compounds is acetate, which is converted directly to methane.

Continuous Culture Theory

Continuous Flow Fermentation

Continuous culture fermentation utilizes a continuous inflow of fresh substrate to maintain the growth of organisms in a culture vessel, and a continuous outflow of spent substrate and organisms from the culture vessel. The key feature of the continuous system is its ability to operate at steady state when substrate is added at a constant flow rate. Single stage reactors with complete mixing (CSTR's) were used in this investigation so that the composition of the effluent was essentially identical to that of the reactor.

The single stage continuous flow reactor which includes a cell separator is shown in Figure 3. In the reactor system shown, the limiting substrate can be supplied either in the liquid influent stream or in an independent influent gas stream.

Kinetic Description

A mathematical description of the single stage completely mixed continuous flow reactor system such as used in biological treatment processes has been reported previously by Agardy *et al*. [38], Stewart [39], Andrews *et al*. [40], and Pearson [41], and is summarized briefly. The following assumptions are used to simplify the development:

1. The yield coefficient and the specific organism decay rate are constant for the range of residence times studied.
2. A constant proportion of the organisms in the culture vessel is viable.

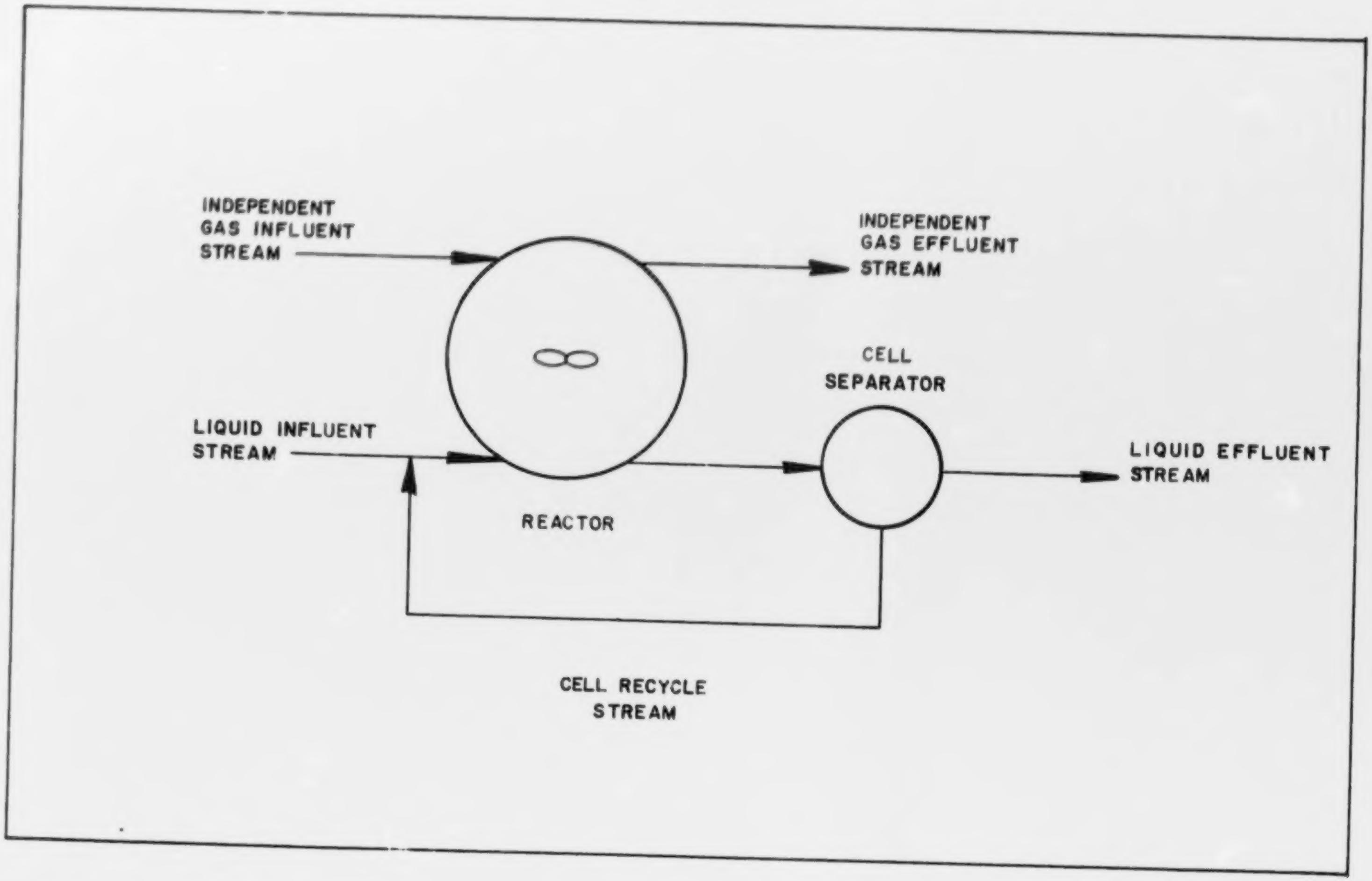

INDEPENDENT GAS INFLUENT STREAM
INDEPENDENT GAS EFFLUENT STREAM
CELL SEPARATOR
LIQUID INFLUENT STREAM
LIQUID EFFLUENT STREAM
REACTOR
CELL RECYCLE STREAM

FIGURE 3. SINGLE-STAGE CONTINUOUS FLOW REACTOR SYSTEM WITH CELL SEPARATION AND CELL RECYCLE

3. The volume of the cell separator is negligible and the total volume of the system is equal to the reactor volume.
4. The mass of organisms in the cell separator is negligible.
5. No active organisms are present in the liquid influent stream.

The substrate removal rate or metabolic coefficient (q) is related to the limiting nutrient concentration (S) by the Michaelis-Menten Equation as follows:

$$q = \frac{\hat{q}S_1}{K_s + S_1} \qquad (6)$$

where:

q = substrate removal rate coefficient, mass of substrate removed per mass of organisms-time

$\hat{q}$ = maximum substrate removal rate coefficient, a constant

K_s = saturation constant, or the substrate concentration at one-half the maximum substrate removal metabolic coefficient, mass per volume

S_1 = concentration of limiting nutrient, mass per volume.

The substrate removal rate can be controlled by a diversity of substances which are growth-limiting nutrients. Postgate and Hunter [42] have used glycerol, lactate, mannitol, magnesium, phosphate, sulfur, and ammonia as limiting nutrients. Pipes [43,44] and Amman and Lynch [45] reported that light and carbon dioxide could limit the growth of algal cultures. Barker [24] controlled the rate of hydrogen assimilation with M. omelianskii by variation of the carbon dioxide concentration.

The substrate removal rate is related to the cell residence time or sludge age (θ_c) by the cell continuity (materials balance) equation as follows:

$$\frac{1}{\theta_c} = Yq - k_d \qquad (7)$$

where:

θ_c = cell residence time

Y = yield coefficient, mass of cells produced per mass of substrate

k_d = specific organism decay rate, time.$^{-1}$

Over a limited range of residence times in which a single group of organisms predominates in the fermentation, Y and k_d are constants. Y and k_d and the Michaelis-Menten constants $\hat{q}$ and K_s give a quantitative description of the kinetic characteristics of the active organisms in the fermentation.

Graphical determination of the constants is shown in Figure 4 (Y and k_d) and in Figure 5 ($\hat{q}$ and K_s).

The specific growth rate of the active organisms can be calculated from the yield coefficient and the substrate removal rate as follows:

$$\mu = Yq \qquad (8)$$

where:

μ = specific growth rate, time.$^{-1}$

The maximum specific growth rate ($\hat{\mu}$) is calculated as below:

$$\hat{\mu} = Y\hat{q} \qquad (9)$$

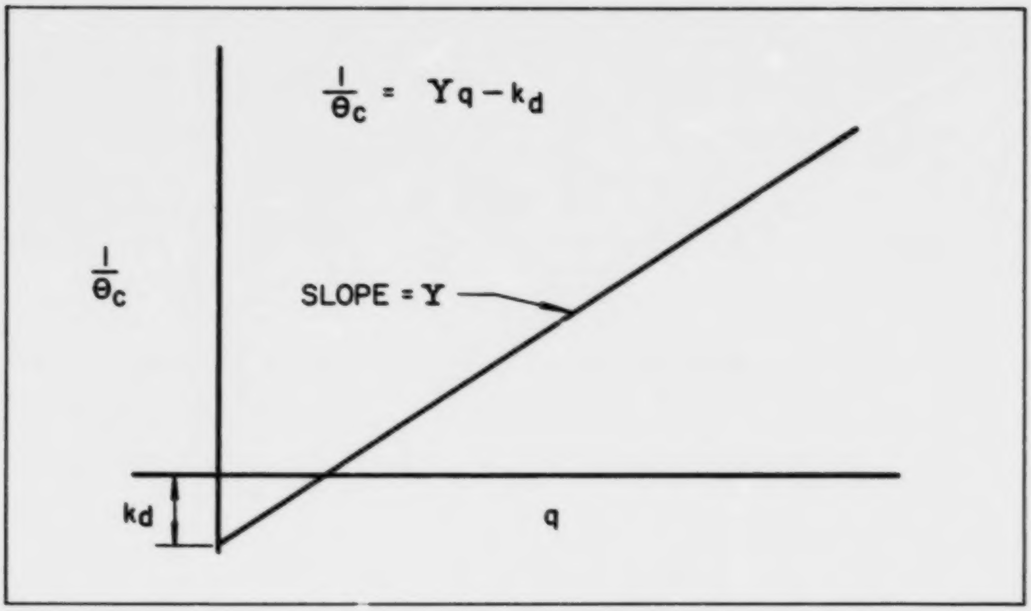

$\frac{1}{\theta_c} = Yq - k_d$
$\frac{1}{\theta_c}$
SLOPE = Y
kd
q

FIGURE 4. DETERMINATION OF Y AND k_d - CELL CONTINUITY EQUATION

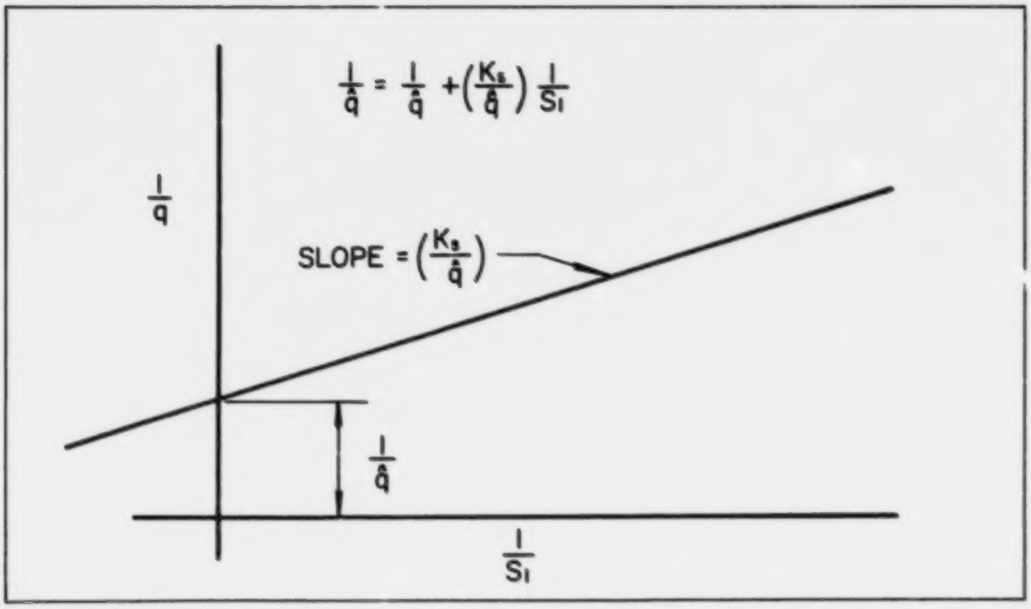

$\frac{1}{q} = \frac{1}{\hat{q}} + \left(\frac{K_s}{\hat{q}}\right)\frac{1}{S_1}$
$\frac{1}{q}$
SLOPE = $\left(\frac{K_s}{\hat{q}}\right)$
$\frac{1}{\hat{q}}$
$\frac{1}{S_1}$

FIGURE 5. DETERMINATION OF $\hat{q}$ AND K_s - MICHAELIS-MENTON EQUATION

The generation time of the active organisms can be calculated from the maximum specific growth rate as follows (when cell division is by binary fission):

$$g = \frac{\ln 2}{\hat{\mu}} \tag{10}$$

where:

g = generation or doubling time of the organism

The critical residence time at which the active organisms wash out of the reactor (θ_w) can be calculated from the maximum specific growth rate by substituting Equation 9 into Equation 7 as below:

$$\frac{1}{\theta_w} = \hat{\mu} - k_d \tag{11}$$

where:

θ_w = critical or washout residence time.

Cell Separation and Recycle

The cell residence time (θ_c) in the reactor vessel is equal to the mass of cells in the reactor divided by the mass of cells leaving the reactor per day, and can be expressed as follows:

$$\theta_c = \frac{\theta X_1}{X_2} \tag{12}$$

where:

θ = hydraulic residence time

X_1 = reactor organism concentration, mass per volume

X_2 = separator effluent organism concentration, mass per volume.

To increase the ratio of the cell residence time and the hydraulic residence time, cell separation devices such as described by Almgren [46], Schroepfer and Ziemke [47,48], and Steffen [49] are used as shown in Figure 3. Cells are separated from the effluent and recycled to the reactor (cell recycle) to increase the reactor organism concentration (X_1) over that in the effluent stream (X_2). The reactor volume required to maintain a mass of cells in a culture vessel is reduced at the increased reactor cell concentration. The increased cell residence time permits a biological unit to be operated at a hydraulic residence time which is less than the critical residence time of the slower growing organisms, without washout of the organisms.

III. EXPERIMENTAL PROCEDURES

The experimental work consisted of two phases: an evaluation of the kinetic constants of a methane bacterium which assimilates hydrogen, and an assessment of the effect of hydrogen on the methane fermentation from volatile acids. The experimental procedures used in the kinetic study and the hydrogen addition study follow.

Hydrogen Enrichment Kinetic Study

Bacterial Culture

An aliquot of an enriched culture of a hydrogen assimilating methane bacterium which had been isolated by Pretorius [50] was made available for the study. The culture had been obtained by recirculating a mixture of 80 percent hydrogen and 20 percent carbon dioxide through a sample of well-digested sludge for about a month. Serial dilutions were made from this culture using the method of Moore [51]. After two weeks of incubation at 37°C, single colonies from the highest dilutions (10^6 to 10^8) were isolated and transferred to a mineral medium supplemented with hydrogen-carbon dioxide mixture. Contrary to the observations of Mylroie and Hungate [22] and Barker [1], suitable growth and methane production were found only in those dilutions in which 500 mg/l of yeast extract had been added to the culture medium.

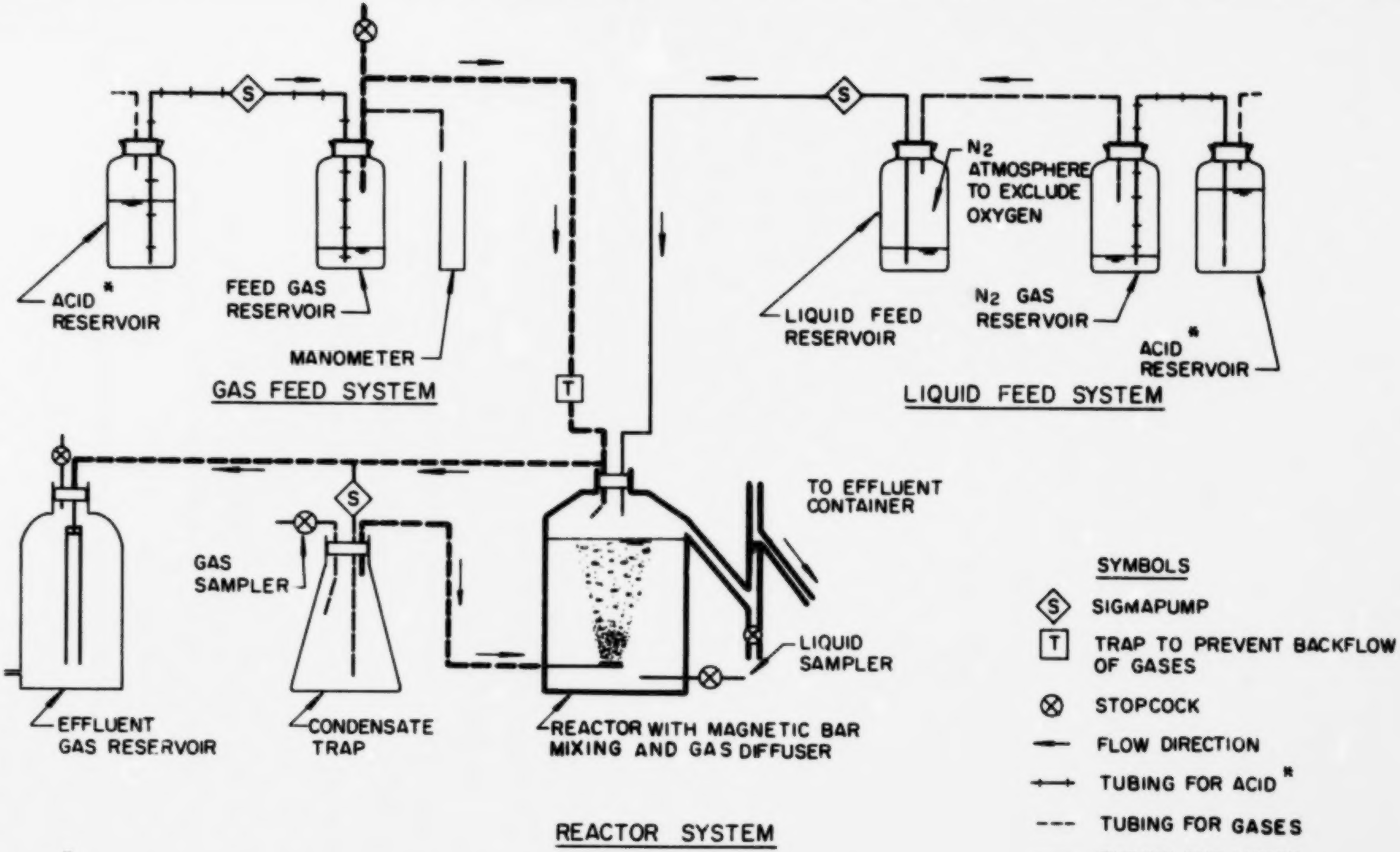
Morphologically the isolated bacterium resembled Methanobacterium formicicum as described by Mylroie and Hungate [22]. The culture was unable to ferment either acetic, propionic, butyric and valeric acids or methanol under a nitrogen atmosphere. During the cultivation of this bacterium, the culture was contaminated by a Clostridium species. Isolates of this Clostridium were unable to form methane from hydrogen and carbon dioxide. The mixed culture was used for the growth and kinetic study as a pure culture was unavailable.

Experimental Apparatus

Two identical fermentation systems (Systems 1 and 2) were operated as enrichment cultures for the hydrogen assimilating methane bacteria. Feed gas and liquid substrate were added intermittently; however, continuous flow operation was approximated by the use of short feed cycle times (10 minute cycles). Gas recirculation through the contents of each reactor was continuous, and each reactor was stirred by magnetic bar mixing during the feed cycle (about 2.5 minutes in each 10 minute interval).

A schematic drawing of one of the two identical systems used in the growth and kinetic characterization is given in Figure 6. Each system consisted of a liquid feed system, a gas feed system, the reactor, an effluent gas reservoir, and a control system. Each of the subunits is described in detail below:

1. Liquid feed system: A sealed four liter flask was used for the liquid substrate reservoir. A nitrogen atmosphere was maintained over the liquid surface in the flask at


ACID * RESERVOIR
FEED GAS RESERVOIR
MANOMETER
GAS FEED SYSTEM
N2 ATMOSPHERE TO EXCLUDE OXYGEN
LIQUID FEED RESERVOIR
N2 GAS RESERVOIR
ACID * RESERVOIR
LIQUID FEED SYSTEM
TO EFFLUENT CONTAINER
GAS SAMPLER
LIQUID SAMPLER
EFFLUENT GAS RESERVOIR
CONDENSATE TRAP
REACTOR WITH MAGNETIC BAR MIXING AND GAS DIFFUSER
REACTOR SYSTEM
SYMBOLS
S SIGMAPUMP
T TRAP TO PREVENT BACKFLOW OF GASES
STOPCOCK
FLOW DIRECTION
TUBING FOR ACID *
TUBING FOR GASES
TUBING FOR LIQUID

* ACID REFERS TO AQUEOUS H_2SO_4 (3.13 % $^{W}/_{w}$) OF SPECIFIC GRAVITY 1.0211

FIGURE 6. ENRICHMENT STUDY CONTINUOUS CULTURE APPARATUS

SYSTEM 1 AND 2

pressures between 760 and 780 mm Hg. Nitrogen reduced the solution of oxygen into the liquid substrate and thereby reduced the amount of oxygen which could enter the reactor from this source. Nitrogen was supplied to the feed flask from a gas reservoir by the gravity transfer of 3.13 percent ($^w/_w$) sulfuric acid solution into the nitrogen reservoir from an acid reservoir.

2. Gas feed system: The gas feed system is shown in Figure 7. The feed gas reservoir was a twenty liter sealed carboy with four lines of Tygon tubing leading to the top of the bottle. The first line was connected to an acid reservoir (H_2SO_4; 3.13 percent $^w/_w$; specific gravity 1.021) through a sigmapump. Acid solution was pumped into the gas reservoir through the first line in order to displace the gas mixture through the second line and into the reactor plenum. A simple device on the second line, labelled "Trap" in Figures 6 and 8, prevented backflow of reactor gases to the gas reservoir. The third line was connected to a manometer and the fourth line was used to refill the reservoir.
3. Reactor system: A glass bottle reactor is shown in Figures 6 and 8. The reactor was constructed from a one liter reagent bottle; an overflow tube was added to maintain a culture volume of 750 ml. A submerged port was provided for the gas diffuser for the liquid sampling tube. The fermentation gases in the reactor were recycled continuously

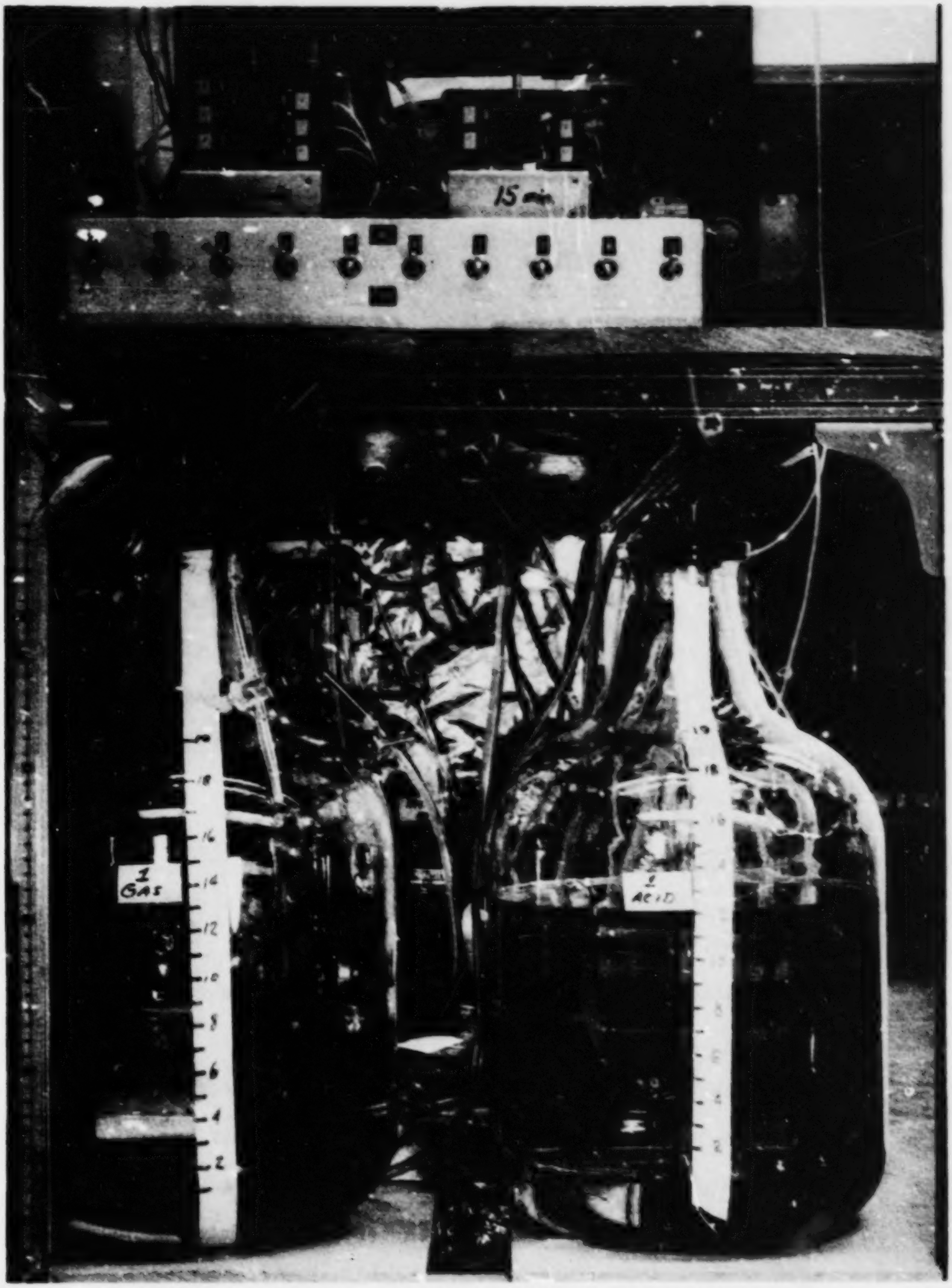

15 min
1 GAS
1 ACID

FIGURE 7. GAS FEED SYSTEM AND PROGRAM TIMER PANEL

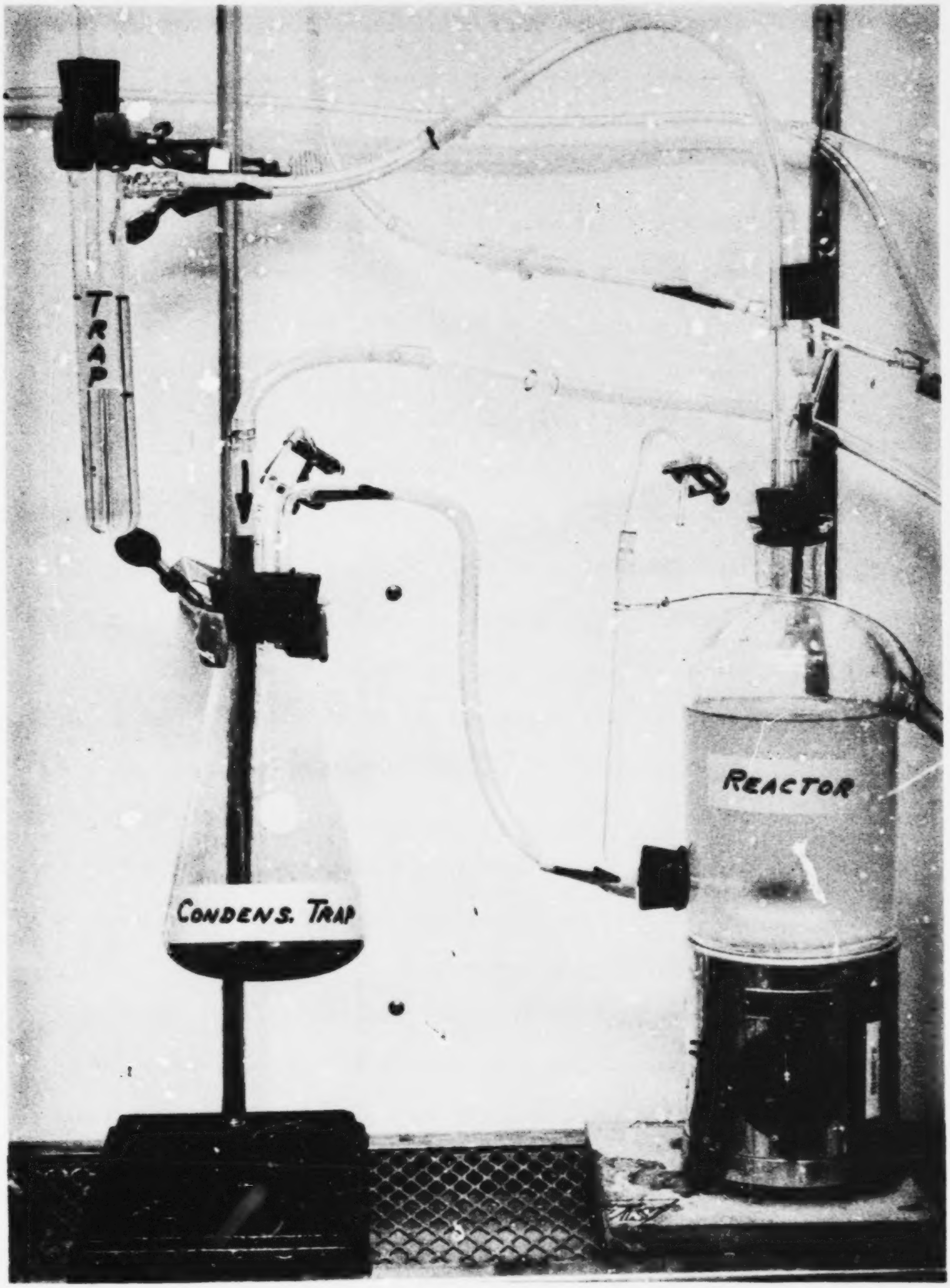

TRAP
CONDENS. TRAP
REACTOR

FIGURE 8. ENRICHMENT STUDY REACTOR.
SYSTEMS 1 AND 2.

from the plenum above the culture through a condensate trap and the gas diffuser back into the culture. Total gas volume undergoing recycle was about 750 ml. A sigma-pump was used to recycle the gases at a rate of 7.5 liters per hour. Gas samples were taken from the condensate trap through a small bore (1 mm ID) tube, and were assumed to be in equilibrium with the dissolved gases due to continuous gas recirculation. Reactor effluents were collected in individual containers.

4. Effluent gas reservoir. Fermentation gases were collected and composited semiweekly over sulfuric acid solution in four liter Mariotte-type gas reservoirs. A description of these units is given in Appendix C.
5. Operation control system. The entire fermentation system was controlled automatically by the program timer panel shown in Figure 7. Cramer multiswitch program timers with cycle times of 10 or 15 minutes were used. The liquid and gas feed pumps to each system were controlled independently, and the pump rates were adjusted by variation of the "on" time fraction at each switch.
6. Tygon tubing was used throughout the system. Although diffusion of gases through the tubing was anticipated, the convenience of Tygon made its use advantageous regardless of the necessity for diffusion corrections. Liquid feed lines were prepared from Tygon Formula SH 50L Tubing which can be autoclaved. Other lines were of Tygon Formula R-3603.

The reactors and gas recycle systems were kept in a constant temperature room at 37 ± 1°C. All other system components were located in the laboratory, which was held at 22 ± 2°C.

Substrate Characteristics

Two independent feed lines were connected to the enrichment culture reactors. One feed stream was the gas mixture which supplied the limiting nutrient, hydrogen, and varying concentrations of carbon dioxide and an inert "filler" gas, nitrogen. The second feed stream supplied essential minerals, growth factors, a buffer and a reducing agent in liquid form and was used to control the dilution rate of the fermentation. The mass rate of hydrogen feed in the gas mixture was proportional to the flow rate of the liquid stream throughout the study.

Substrate Gas.--The substrate gas mixtures were designed to supply hydrogen and carbon dioxide, with hydrogen as the limiting nutrient. Four moles of hydrogen react stoichiometrically with one mole of carbon dioxide to give methane (Equation 1). Consequently, the molar ratio of hydrogen to carbon dioxide in the substrate gas was maintained at less than four except at the lowest cell residence times, when a mole ratio of four was maintained. Because hydrogen and carbon dioxide may be used in reactions other than methane formation by Equation 1, the molar ratio of four ($H_2:CO_2$) in the substrate gas is an estimate of the appropriate ratio needed to ensure that hydrogen is the limiting substrate.

Hydrogen and carbon dioxide concentrations in the gas mixture were greater at the lower cell residence times than at the higher, as shown in Figure 9. This variation permitted the mass rate of hydrogen-carbon dioxide supply to be increased as the liquid flow rate increased, with only minimal change in the pumping rate of the gas mixture. The nitrogen concentration in the gas mixture decreased with decreasing cell residence times as illustrated in Figure 9.

Substrate gas mixtures were prepared from commercial grade, stock cylinders of each gas component. Each mixture was analyzed in duplicate immediately upon preparation. Numerous subsequent analyses of gas mixtures yielded gas compositions which varied within ± 1 percent of the original analysis.

Liquid Substrate.--The liquid substrate used was similar to that employed by Pretorius [50], and the composition and general characteristics of the substrate are reported in Tables III and IV respectively. An analysis of the yeast extract employed is presented in Appendix A. No suspended solids were present in the feed stream; thus the volatile suspended solids (VSS) in the reactor were used as a measure of the organisms in the system.

The liquid substrate was supplied in proportion to the gas substrate, and contained sufficient phosphate buffer to maintain the pH in the reactors between 6.7 and 7.3. The ammonium salt was added in excess. Mercaptoacetic acid was supplied as a reducing agent for the methane bacteria, and resazurin was added as a color indicator of the oxygenation of the liquid substrate. Yeast extract provided essential growth factors.

Distilled water was used in media preparation. Fresh substrate was mixed at least biweekly and autoclaved before use to minimize

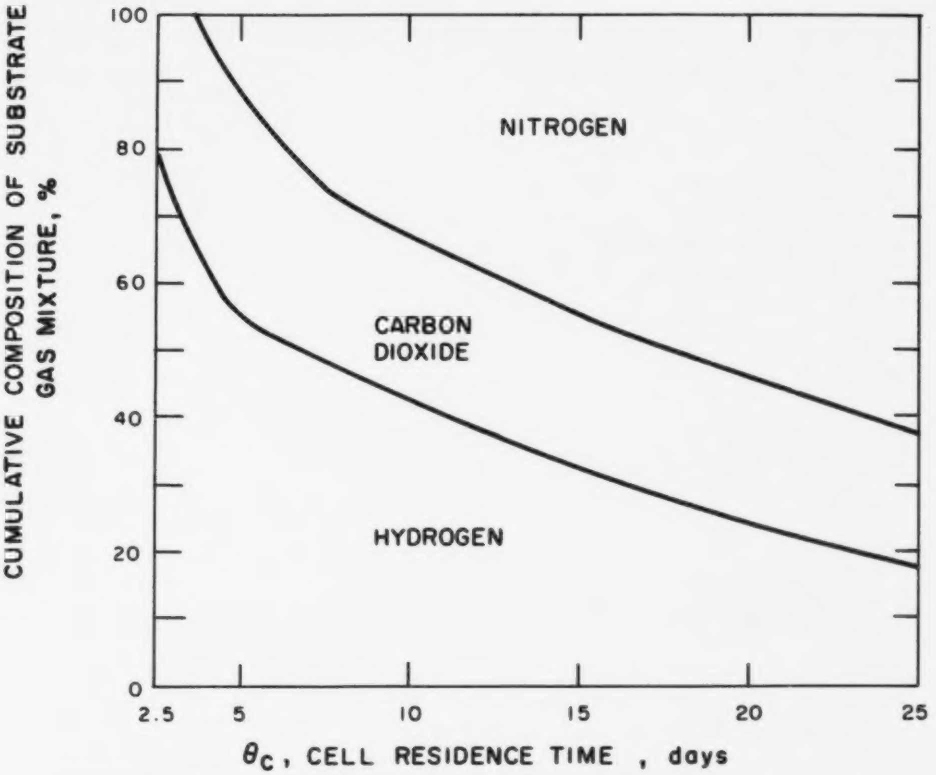


FIGURE 9. COMPOSITION OF SUBSTRATE GAS MIXTURE WITH VARIATION OF CELL RESIDENCE TIME

TABLE III

LIQUID SUBSTRATE COMPOSITION FOR ENRICHMENT CULTURE FERMENTATIONS [50]

Item	Concentration mg/liter
Inorganics	
K_2HPO_4	1,500
NH_4Cl	500
Na_2CO_3	24
$CaCl_2 \cdot 2H_2O$	10
$MgCl_2 \cdot 6H_2O$	10
$FeCl_3 \cdot 6H_2O$	2
$MnSO_4 \cdot 4H_2O$	1
Na_2MoO_4	1
Organics	
Mercaptoacetic acid	500
Yeast extract	500
Resazurin	2

TABLE IV

LIQUID SUBSTRATE CHARACTERISTICS FOR ENRICHMENT CULTURE FERMENTATIONS

Item	Magnitude
Volatile Solids	1,000 mg/ℓ
Total Solids	2,860 mg/ℓ
Suspended Solids	Negligible
pH	7.82
Alkalinity	460 mg/ℓ
Total Nitrogen	190 mg/ℓ
Ammonia Nitrogen	140 mg/ℓ
COD (Dichromate reflux)	1,340 mg/ℓ
Acetic Acid	10 mg/ℓ
Other Volatile Acids	Negligible

the possibility of bacterial degradation. Batch culture tests with autoclaved substrate showed that heat-labile compounds (such as tryptophane) lost on autoclaving were not rate limiting.

Analytical Methods

The sample processing procedure and analytical methods used are shown diagrammatically in Figure 10. Material balances on the reactors were conducted semiweekly. Duplicate analyses were made both at the beginning and the end of each semiweekly balance period (a total of four analyses) except for volatile acids and ammonia nitrogen, which were determined once weekly. Each set of analytical results for volatile suspended solids, reactor hydrogen concentration, and reactor soluble COD was averaged for every balance period. The average value was reported as representative of the balance period.

Selected details and modifications of the analytical methods employed in the study are noted as follows.

Suspended Solids.--The centrifugation technique described by Almgren [46] was used. Twenty-five ml samples were withdrawn from each reactor during an interval of intensive mixing. Two 10 ml aliquots were prepared for analysis from each sample. Sample sizes were limited to 25 ml because the reactor size was only 750 ml. The quantity of cells collected in the centrifuge tube (1-2 mg) was determined to the nearest 0.05 mg. The coefficient of variation C_v (standard deviation divided by mean) of the dry weight of 10 replicate analyses of a single sample was six percent.

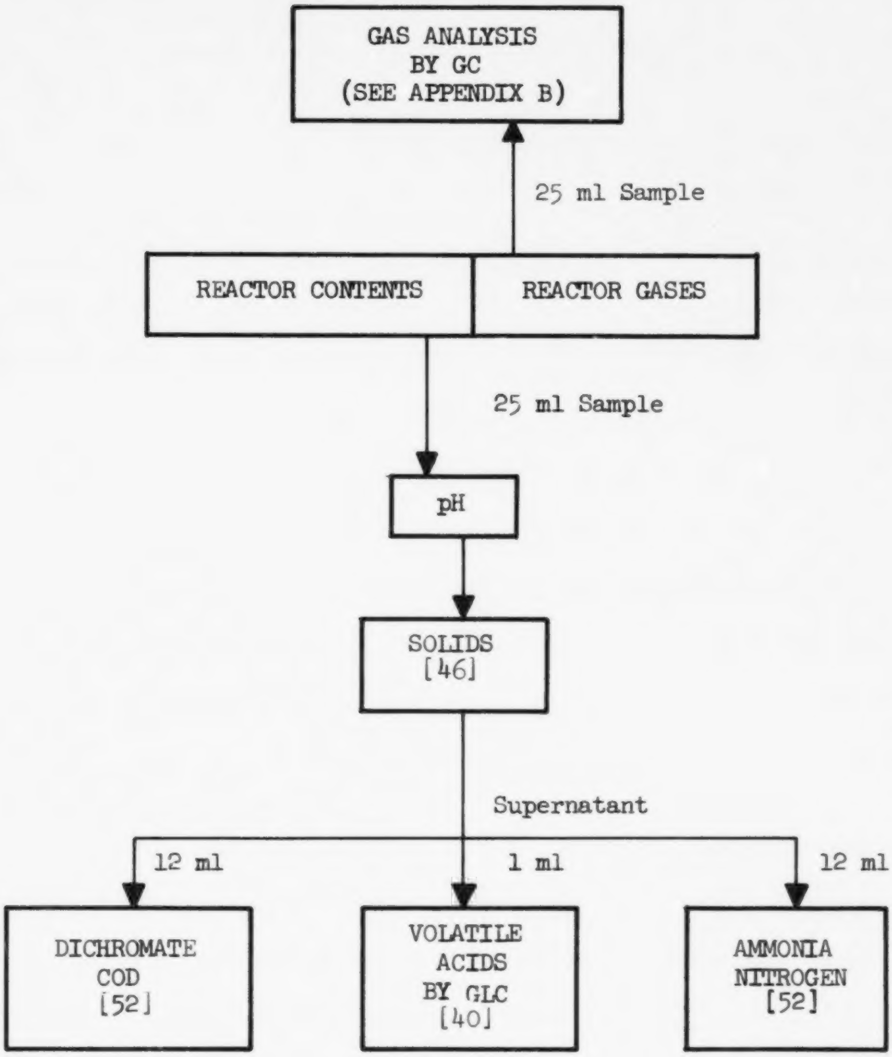

GAS ANALYSIS
BY GC
(SEE APPENDIX B)
25 ml Sample
REACTOR CONTENTS
REACTOR GASES
25 ml Sample
pH
SOLIDS
[46]
Supernatant
12 ml
1 ml
12 ml
DICHROMATE
COD
[52]
VOLATILE
ACIDS
BY GLC
[40]
AMMONIA
NITROGEN
[52]

FIGURE 10. ANALYSIS OF REACTOR GASES AND CONTENTS ENRICHMENT STUDY

Gas Analyses.--Gas solid chromatography was selected as a rapid and accurate mean of gas analysis for the study [53, 54, 55]. Minimal volumes of sample gas (10 ml) were required for analysis. A description of the chromatography equipment used and the procedure are given in Appendix B. The coefficients of variation of the analysis ranged from a minimum of 0.21 percent (N_2) to a maximum of 1.18 percent (CH_4).

Each gas sample was analyzed in duplicate for hydrogen (on nitrogen carrier gas), and CO_2, O_2, N_2 and CH_4 (on helium carrier gas). A hydrogen standard was analyzed with every sample that contained hydrogen. It was unnecessary to prepare standards for CO_2, C_2, N_2, and CH_4 every time, because the ratios of the values for the calibration factors of these gases were constants. The values of these calibration factors varied equally ± 2 percent with daily changes in ambient temperature; thus the concentrations of CO_2, O_2, N_2, and CH_4 from each analysis could be normalized proportionately to total 100 percent when added to the hydrogen concentration of the sample. The hydrogen concentration did not require correction because it was analyzed each time against a new standard. Standards for CO_2, O_2, N_2, and CH_4 were prepared monthly.

Operation

Startup.--The two continuous flow reactors were charged initially with 500 ml each of a batch enrichment culture of the hydrogen assimilating methane bacterium. The development of the inoculum biomass from the original aliquot of Pretorius [50] required about one month, during which a mixture of 80 percent hydrogen - 20 percent carbon dioxide was recirculated through the culture. The reactors were flushed with at least five volumes of 80 percent hydrogen - 20 percent carbon dioxide to remove oxygen before the enrichment culture was added.

The feeding of substrate gas and liquid mixtures was started immediately after the reactors were charged. After the culture levels in the reactor reached overflow volume (750 ml), each reactor was operated for a six-week period (about two cellular and hydraulic residence times) to check out operational and analytical procedures and to establish steady state conditions. Steady state was defined as follows: the repetition of the reactor hydrogen concentration (partial pressure, mm Hg), and the unit hydrogen removal rate (mg H_2-COD/mg VSS-day) within ± 10 percent during three or more consecutive semiweekly balance periods at the same cell residence time. These variables represent S, q, and θ_c in the growth and kinetic equations (Equations 6 and 7).

Material Balances.--Material balances for each system were prepared by measurement of the following characteristics:

1. Weight of liquid substrate fed.
2. Weight of substrate gas ged.
3. Manometer differential (mm manometer fluid) at the substrate gas reservoir.
4. Fermentation gas production (from the volume of acid displaced from the effluent gas reservoir).
5. Weight of liquid effluent.

6. Temperature and pressure of the reactor and of the external system components.

Sampling and Analysis.--Reactor liquid effluents and effluent fermentation gases were composited during each balance period, and a complete set of analyses was made at the end of the balance period. Twenty-five ml gas and liquid samples were collected from the reactor at the beginning and end of each balance period. Gas samples were collected first because removal of the liquid sample caused substrate gas to enter the reactor. The averages of the initial and final reactor sample data for a balance period were used to calculate the reactor kinetic variables S, q, and θ_c to which the steady state criteria were applied.

Routine maintenance included daily cleaning of the internal reactor walls to minimize development of wall growths of bacteria interfering with the kinetic analysis. The cleaning was accomplished by pulling a teflon-covered mixing bar over the reactor walls with an external magnet.

COD Balance Calculations

Material balances for kinetic analysis of the enrichment system were developed by equating the COD inputs (hydrogen COD and soluble COD) of each reactor with the COD outputs (hydrogen COD, methane COD, soluble COD and cellular COD) in each balance period. The net removal of hydrogen and the total production of methane were quantitated by a balance of the influent, reactor sample, and effluent gases. Because Tygon tubing was used for the influent and effluent gas lines, a diffusion correction for loss of hydrogen and methane was included in the gas balance.

Additional data processing was required before the gas and COD balances and kinetic analyses could be developed. Special procedures for additional calculations are described below.

Gas Composition. Each complete gas analysis consisted of duplicate chromatograms with peak areas for hydrogen (nitrogen carrier gas) and CO_2, O_2, N_2, and CH_4 (helium carrier gas). The peak areas were determined by automatic area integration, and multiplied by the appropriate calibration factor to give the percent composition for each component. The concentrations of CO_2, O_2, N_2, and CH_4 were normalized proportionately to equal 100 percent when added to the hydrogen concentration, as discussed previously.

Gas Balance.--Influent, reactor sample, and effluent gas volumes were corrected to dry condition at 0°C and 760 mm Hg, and converted into volume flow rates. The influent (substrate gas) volume flow rate was calculated from the weight of acid pumped as follows:

Influent gas flow rate, liters per day (dry, 0°C, 760 mm Hg)

$$= \frac{\Delta W}{\rho} \; \frac{273}{760} \; \frac{(\bar{P} - P_v)}{\bar{T}t} \qquad (13)$$

where:

W = Weight of the acid reservoir for each gas feed system

ρ = Density of aqueous 3.13 percent ($^w/_w$) H_2SO_4 solution, 1.021 g/cu cm.

$\bar{P}$ = Average sum of the total (atmospheric plus manometric differential) pressures in the substrate gas reservoir at the beginning and end of a balance period, mm Hg.

P_v = Vapor pressure of water over the container liquid at temperature $\bar{T}$, mm Hg.

$\bar{T}$ = Average of temperatures at the beginning and end of a balance period, °K.

t = Duration of balance period, days.

The reactor sample gas flow rate was calculated as follows:

Reactor sample gas flow rate, liter/day (dry, 0°C, 760 mm Hg)

$$= (.025 \frac{273}{760} \frac{(P-P_v)}{Tt} \qquad (14)$$

where:

P = Atmospheric pressure, mm Hg

T = Temperature, °K

Effluent fermentation gases were held at slightly negative pressures in Mariotte-type gas collectors as described in Appendix C. Volume flow rates were calculated from the volume of liquid displaced as follows:

Effluent gas flow rate, liters/day (dry, 0°C, 760 mm Hg)

$$= V_L \frac{273}{760} \frac{(P-P_V-0.0734\,\rho h)}{Tt} \qquad (15)$$

where:

V_L = Volume of liquid displaced by fermentation gases, liters

h = Height of the column of liquid suspended above the liquid outlet level, mm.

Volume flow rates of CH_4, H_2, and CO_2 in the influent, reactor sample and effluent gas streams were calculated from the above volume flow rates and the composition of each gas stream. These data represent the flow rates of CH_4, H_2, and CO_2 from the feed gas reservoir, the reactor gas samples, and into the effluent gas reservoir respectively, before correction for diffusion losses. The results are summarized in Tables XXV and XXVI of Appendix E.

Calculations showed that the quantities of hydrogen and methane which were removed from the reactor as dissolved gases in the reactor effluent were less than one percent of respective amounts removed from or by the gas stream. Thus dissolved gases were neglected in the gas balances.

Diffusion Losses

The diffusion loss of hydrogen in the influent gas lines and of hydrogen and methane in the effluent gas lines was expected with the use of Tygon tubing (Formula R-3603). To incorporate a diffusion correction into the gas balance, the permeabilities of hydrogen and methane through the Tygon gas lines were measured in the actual systems (Figure 6) used for the kinetic study. A theoretical development of the correction for diffusion loss and a description of the procedure are given in Appendix D.

Each of the influent and effluent gas lines of the two systems consisted of 10 foot lengths of tubing which measured 0.318 cm ID by 0.159 cm wall thickness. The steady state volume flow rate of hydrogen diffusion for each length of tubing was estimated as follows (from Treybal [56], as described in Appendix D):

$$(V_D)_{H_2} = 0.221 \text{ liters/day - atm hydrogen} \quad (16)$$

where:

$(V_D)_{H_2}$ = volume of hydrogen (0°C and 760 mm Hg) diffusing per day per atmosphere of hydrogen.

The expression for methane is as follows:

$$(V_D)_{CH_4} = 0.0676 \text{ liters/day - atm methane} \quad (17)$$

where:

$(V_D)_{CH_4}$ = volume of methane (at 0°C and 760 mm Hg) diffusing per day per atmosphere of methane.

The diffusion loss of influent hydrogen was calculated by Equation 16; the partial pressure of hydrogen in the substrate gas mixture was used for the pressure differential. Diffusion losses of hydrogen and methane in the effluent lines were calculated by Equations 16 and 17, respectively. The pressure differential in the effluent lines was assumed to equal the average of the partial pressures of each gas in the reactor and in the effluent reservoir.

The diffusion losses for influent and effluent hydrogen and effluent methane are presented in Tables XXVII and XXVIII of Appendix E. Data reported in these tables indicate the magnitude of the diffusion losses to be as follows:

1. Eight to 22 percent of influent hydrogen.
2. Twenty-six to 61 percent of effluent hydrogen.
3. Seven to 14 percent of methane produced.

The data also show that the percent significance of the loss decreased at higher gas flow rates.

The magnitude of these losses confirmed the need for incorporating a suitable correction for diffusion losses into the COD balance calculations.

Conversion Factors.--After adjustment for diffusion losses, all gas flow rates were converted to mass flow rates (COD basis) by the factors presented in Table V.

TABLE V

GAS COD CONVERSION FACTORS

Gas	Moles COD / Mole Gas	mg COD / Mole Gas	mg COD / Liter Gas
H_2	0.5	16,000	714
CH_3	2.0	64,000	2857

Volatile suspended solids were converted to cell COD by the conversion factor of 1.42 g COD per g volatile suspended solids, as discussed by McCarty [57]. Gas balance data, cellular COD production, and input and output soluble COD are presented in the COD balance in Appendix E.

Kinetic Evaluation

Approach.--The kinetic evaluation of an enrichment organism is limited by the presence of a contaminant organism, the growth and metabolic activities of which are frequently inseparable from those of the enrichment organism. The uptake of hydrogen by the hydrogen assimilating methane bacterium was anticipated to be the major type of metabolic activity in this enrichment culture investigation. However, hydrogen may also have been used by acetate-synthesizing Clostridia species or by other organisms. For this reason, the uptake of hydrogen may not reflect only the activity of hydrogen assimilating methane bacteria. Likewise, the removal of soluble COD may result from metabolic activity or cell synthesis by contaminant organisms. For example, several two or three carbon organic acids (such as acetic acid) which may be metabolic byproducts of contaminant organism activity are also methane carbon precursors (Scherfig [37]). Consequently, the conversion of soluble COD (organic acids) to methane would not only remove soluble COD from the reactor contents, but also would generate methane from a source other than the reduction of carbon dioxide with hydrogen.

Despite these limitations, the rate coefficients for hydrogen COD and total COD (hydrogen plus soluble COD) removal appear feasible for kinetic assessment of the enrichment cultures. The units of these coefficients are: mass of hydrogen COD and total COD removed respectively per mass of organisms in the reactor per unit time.

Techniques are not available to permit ready quantitation of the enriched methane organisms apart from the contaminants. Thus the accuracy of a kinetic description which uses organism population (VSS) as the denominator of a rate coefficient increases as the ratio of the methane bacteria to the total population increases. Nonetheless, the rate coefficients for hydrogen and total COD removal comprise the only available rational bases for comparison of the substrate uptake in the hydrogen enrichment fermentation with that of other methane producing systems.

The activity of methane bacteria can also be measured by the methane rate coefficient (mass of methane produced per mass of organisms per time). Methane rate coefficients are available for continuous culture enrichment fermentations of acetate, formate, propionate, and methanol. These coefficients are not specific for active methane bacteria; however, they do permit comparison of methane rate coefficients obtained in previous research with the results of this study.

Calculations.--Substrate COD removal can be defined as follows:

1. H_2-COD: the removal of hydrogen from the independent gas stream. Less than one percent of the hydrogen removed was due to dissolved hydrogen leaving the reactor in the liquid effluent; thus only hydrogen removed from the gas stream was considered routinely.
2. Soluble COD: the removal of soluble COD in the form of dissolved organic matter. Although hydrogen was available biologically only in the dissolved phase, hydrogen is excluded from this category and included under H_2-COD.
3. Total COD: the sum of COD removal as H_2-COD and soluble COD.

The substrate removal rate for H_2-COD was calculated as follows:

$$q_{H_2} = \frac{G_0 - G_1}{X_1 V} \tag{18}$$

where:

q_{H_2} = hydrogen removal rate coefficient, mg H_2-COD removed per mg VSS-day

G_0 = influent hydrogen flow rate, mg H_2-COD per day
G_1 = effluent hydrogen flow rate (from both effluent gas and gas sample streams), mg H_2-COD per day.
V = reactor volume

The soluble COD removal metabolic coefficient was calculated as below:

$$q_L = \frac{F_0(S_0)_L - F_1(S_1)_L - F_s(S_1)_L}{X_1 V} \qquad (19)$$

where:

q_L = soluble COD removal rate coefficient, mg soluble COD removed per mg VSS-day
F_0 = liquid influent flow rate, liters per day
F_1 = reactor effluent flow rate, liters per day
F_s = reactor sample flow rate, liters per day
$(S_0)_L$ = influent soluble COD concentration, mg COD per liter
$(S_L)_L$ = reactor and effluent soluble COD concentration, mg COD per liter

The total COD removal was equal to the sum of H_2-COD and soluble COD removal as follows:

$$q_T = q_{H_2} + q_L \qquad (20)$$

where:

q_T = total COD removal rate coefficient, mg total (H_2 plus soluble) COD removed per mg VSS-day.

Cell recycle was not used in the enrichment study; consequently, the cell residence time and hydraulic residence time are equal. The cell residence time was calculated as follows:

$$\theta_c = \frac{V}{F_1 + F_s} \tag{21}$$

The sum $(F_1 + F_s)$ is the total liquid outflow rate of the system.

The substrate COD loading coefficients and the COD removal efficiencies based on hydrogen, soluble, and total COD respectively were calculated as follows:

$$(L_c)_{H_2} = \frac{G_0}{X_1} \tag{22}$$

$$E_{H_2} = 100 \frac{G_{H_2}}{(L_c)_{H_2}} \tag{23}$$

$$(L_c)_L = \frac{F_0 (S_0)_L}{X_1 V} \tag{24}$$

$$E_L = 100 \frac{q_L}{(L_c)_L} \tag{25}$$

$$(L_c)_T = (L_c)_{H_2} + (L_c)_L \tag{26}$$

$$E_T = 100 \frac{q_T}{(L_c)_T} \tag{27}$$

where:

$(L_c)_{H_2}$ = hydrogen COD loading coefficient, mg H_2-COD per mg VSS-day

E_{H_2} = efficiency of hydrogen COD removal, percent

$(L_c)_L$ = soluble COD loading coefficient, mg soluble COD per mg VSS-day

E_L = efficiency of soluble COD removal, percent

$(L_c)_T$ = total COD loading coefficient, mg total COD per mg VSS-day

E_T = efficiency of total COD removal, percent.

The microbiological constants Y, k_d, $\hat{q}$, and K_s were calculated from the steady state growth and kinetic characteristics as indicated in Figures 4 and 5. The hydrogen partial pressure in the reactor, defined as $(S_1)_G$, was used as the growth limiting nutrient in the Michaelis-Menten equation (Equation 6). The methane production rate coefficient was defined by the term q_{CH_4}, in units of mg CH_4-COD produced per mg VSS-day. This rate coefficient was used to compare the methane productivity of the enrichment cultures with other methane-producing fermentations.

Hydrogen Supplementation

Experimental Apparatus

Two continuous flow reactor systems were used to investigate the effect of hydrogen on the methane fermentation of volatile acids (Systems 3 and 4). The flow sheets for these systems were similar to those described previously for the hydrogen enrichment study (Figure 6), but with two exceptions: Lucite reactors (5 liter capacity) shown in Figure 11 were used, and cell recycle was provided to maintain the slower growing species in the reactors.

The liquid contents of each reactor could be recirculated by sigmapump as shown in Figure 12. The cross-recirculation arrangement was used to mix the contents of each reactor system with the other to ensure culture homogeneity prior to an experiment; the recirculation arrangement was used to mix separately the cultures in each reactor during an experiment. The circulation rate was 1.7 liters per hour in both reactors, which was equivalent to the

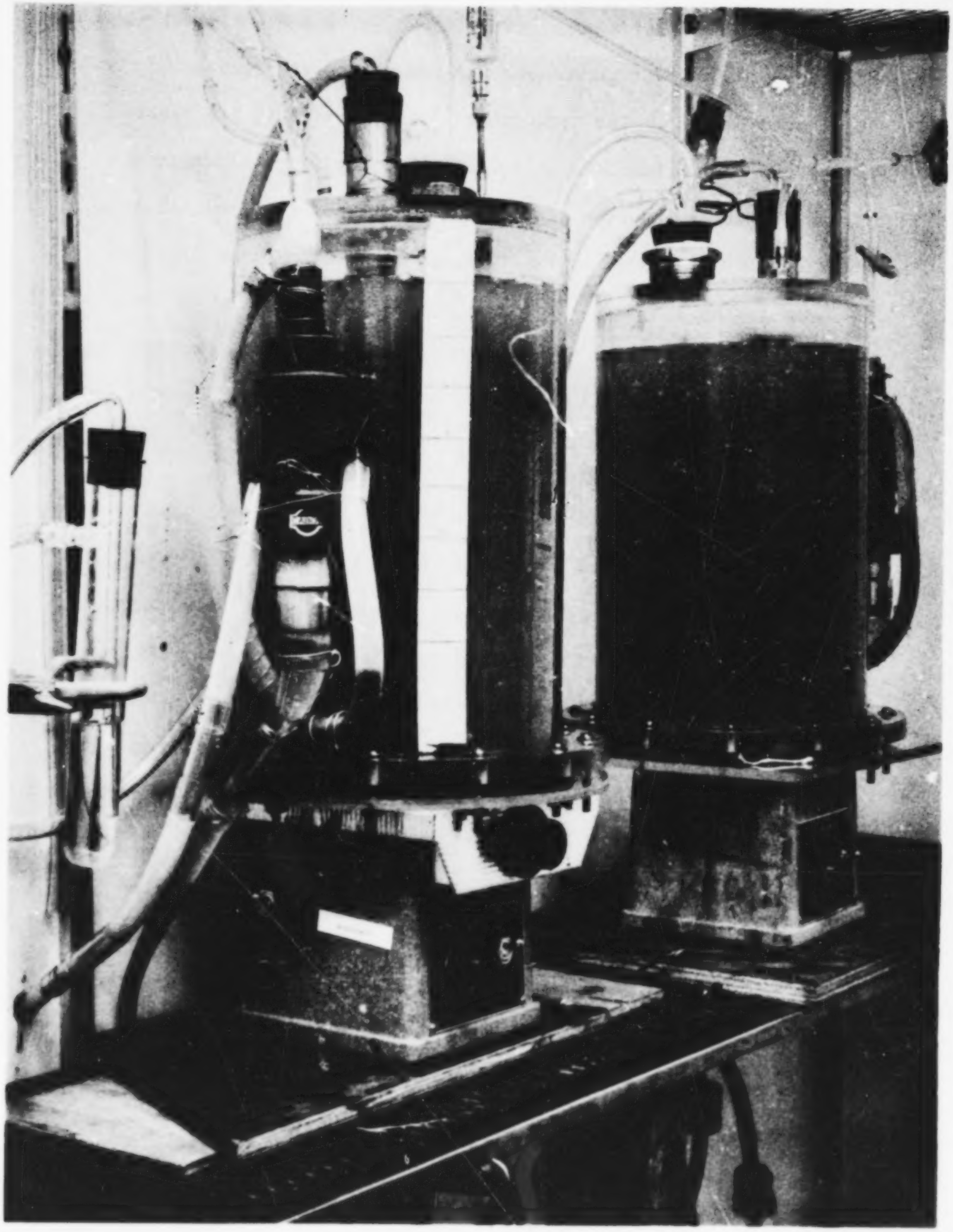


FIGURE 11 HYDROGEN SUPPLEMENTATION STUDY REACTORS. SYSTEMS 3 AND 4

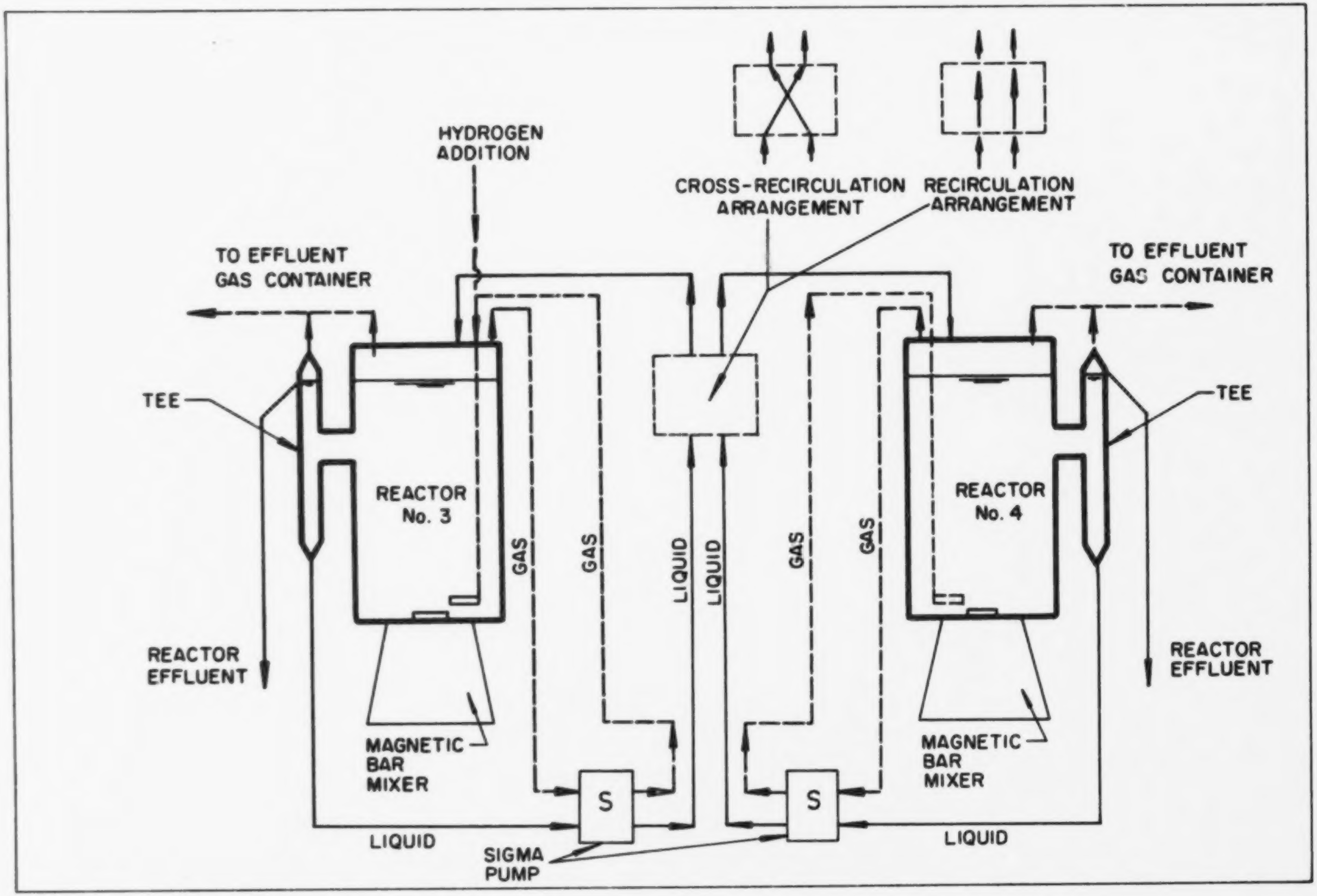

HYDROGEN ADDITION
CROSS-RECIRCULATION ARRANGEMENT
RECIRCULATION ARRANGEMENT
TO EFFLUENT GAS CONTAINER
TO EFFLUENT GAS CONTAINER
TEE
TEE
REACTOR No. 3
REACTOR No. 4
GAS
GAS
LIQUID
LIQUID
GAS
GAS
REACTOR EFFLUENT
REACTOR EFFLUENT
MAGNETIC BAR MIXER
MAGNETIC BAR MIXER
S
S
LIQUID
LIQUID
SIGMA PUMP

FIGURE 12. GAS AND LIQUID RECIRCULATION FLOW SHEET FOR HYDROGEN SUPPLEMENTATION STUDY

SYSTEM 3 AND 4

daily circulation of eight culture volumes. The reactor contents were stirred for 2 1/2 minutes every 10 minutes by the magnetic bar mixers.

The cell recycle apparatus and liquid effluent overflow were part of the circulation system. Liquid effluent flowed over a weir in the upper arm of the tee at the side of each reactor, as shown in Figure 12. The gas pressures in the plenum of the tee and in the reactor were equal, which permitted the level of the reactor contents to be controlled by the weir level. The upper arm of each tee acted as an upflow clarifier for separation of settleable solids from the liquid effluent. The settled solids (cells) could be recycled to the reactor.

The surface loading rate in the upper arm of the tee equalled about 18 gpd/sq. ft. at a hydraulic residence time of 25 days, whereas the recirculation rate of 1.7 liters per hour equalled a rate of 3,500 gpd/sq. ft. in the lower arm. The volume of the tee was 90 cu. cm, or less than two percent of the total reactor volume.

Fermentations gases were recycled within each reactor as shown in Figure 12. The gas volume in each reactor plenum was 500 ml, and the gas circulation rate was 2 liters per hour.

Systems 3 and 4 were controlled by the program timer panel described previously for the enrichment study. The reactors were maintained at 37 ± 1°C in a constant temperature room, and the substrate containers were refrigerated at 5°C.

Substrate Characteristics

Substrate Gas.--The substrate gas mixture was prepared from stock cylinders of commercial grade hydrogen. Duplicate analyses of the reservoir gas were made after each refilling of the reservoir to determine the exact hydrogen concentration. The reservoir hydrogen concentration ranged from 96 to 98 percent.

Liquid Substrate.--The synthetic liquid substrate used was similar to that of Agardy *et al* [38]. The general organic composition of the substrate is given in Table VI, and its chemical characteristics are described in Table VII. The substrate concentration was reduced during the first weeks of the study to maintain the soluble COD load coefficient $(L_c)_L$ less than 1 mg soluble COD per mg VSS-day. As the VSS of the reactors increased, the substrate concentration could be increased to that of Tables VI and VII.

Tap water was used in preparation of the soluble substrate. Fresh substrate was prepared weekly and refrigerated at 5°C to prevent bacterial degradation. Liquid substrate feed lines were rinsed weekly with 1 *N* NaOH, followed by distilled water, to prevent bacterial growth.

Analytical Methods.--The analytical methods were identical with those described previously for the enrichment study.

Operation

The two reactors were charged initially with 2 1/2 liters of screened (200 mesh) digesting municipal sludge from the Stege Sanitary District plant in Richmond, California, and 2 1/2 liters of warm

TABLE VI

LIQUID SUBSTRATE COMPOSITION FOR HYDROGEN SUPPLEMENTATION STUDY

Item	Concentration g/liter
Tryptone (Difco)	5.18
Beef Extract (Difco)	1.24
Yeast Extract (Difco)	0.31
Dextrose	2.59

TABLE VII

LIQUID SUBSTRATE CHARACTERISTICS FOR HYDROGEN SUPPLEMENTATION STUDY

Item	Magnitude
Volatile Solids	6,320 mg/ℓ
Total Solids	7,020 mg/ℓ
Suspended Solids	Negligible
pH	6.91
Alkalinity	270 mg/ℓ
Total Nitrogen	740 mg/ℓ
Ammonia Nitrogen	10 mg/ℓ
COD (Dichromate reflux)	9,620 mg/ℓ
Acetic Acid	40 mg/ℓ
Other Volatile Acids	Negligible

tap water. Each reactor was flushed with 10 volumes of nitrogen before it was charged. The systems were operated with continuous liquid substrate feed and gas and liquid circulation for about one hydraulic residence time (25 days) before material balances were initiated.

The operating program consisted of four six-week phases, each nearly two hydraulic residence times in length. The cross-recirculation arrangement (Figure 12) was used during the first six weeks of operation to ensure similarity of the contents in the two reactors under similar liquid feed conditions. During the second six-week period, the two systems were separated and hydrogen was added to System 3. The third phase was used to observe the changes in System 3 after addition of hydrogen had been terminated. Hydrogen was added to System 4 during the fourth six-week period, and both systems were operated an additional three weeks after hydrogen addition to System 4 was terminated, for a total operating period of 27 weeks. The liquid feed rates were identical and constant for both reactors throughout the study.

Material balances for kinetic analysis were conducted on a weekly basis. At the beginning and end of each balance period, 40 ml liquid samples were collected from the reactor. The averages of the initial and final data were used to represent the average condition of the reactors during the balance period.

The internal surfaces of the reactors were cleaned weekly to minimize wall growth as described previously.

Calculations

The use of cell recycle necessitated the calculation of the cell residence time by a modification of Equation 21 as follows:

$$\theta_c = \frac{VX_1}{F_s X_1 + F_1 X_2} \qquad (28)$$

where:

X_2 = separator effluent organism concentration, mass per volume.

The remaining calculations were similar to those described previously for the enrichment study.

IV. HYDROGEN ENRICHMENT KINETIC STUDY

Results

Two identical continuous culture systems (Systems 1 and 2) were studied to determine kinetic constants for the hydrogen assimilating culture of methane bacteria described previously. Steady state operation was maintained for data collection, and steady state was defined as the condition which was characterized by hydrogen concentration $(S_1)_G$ and hydrogen removal rate (q_{H_2}) variations within ± 10 percent during three or more consecutive semi-weekly balance periods at a given cell residence time.

The substrate removal and loading rate coefficients q_{H_2}, q_T, $(L_c)_{H_2}$, $(L_c)_T$, cell concentrations (X_1), and cell residence times (θ_c) of all semiweekly balance periods for Systems 1 and 2 are summarized in Table VIII. These results were obtained or computed from data summarized in the following tables of Appendix E:

- Table XXIV: (Flow rate, substrate concentration, and cell concentration data)
- Tables XXVII and XXVIII: (Influent H_2 flow rates and effluent H_2 and CH_4 flow rates, adjusted for diffusion losses)
- Table XXIX: (Influent H_2-COD and soluble COD flow rates and effluent H_2-COD, soluble COD, CH_4-COD, and cell-COD flow rates.)

TABLE VIII

GROWTH AND KINETIC DATA - ENRICHMENT STUDY

Balance Period	H_2-COD removed mg/day	Soluble COD removed mg/day	X_1 mg/ℓ [a]	θ_c days [b]	q_{H_2} [c]	q_T [d]	$(L_c)_{H_2}$ [e]	$(L_c)_T$ [f]	E_{H_2} % [g]	E_T % [h]
				System 1						
1	291.3	13.2	122	7.61	3.18	3.32	3.80	5.25	83.6	63.2
2	290.6	11.5	118	7.02	3.28	3.41	3.93	5.66	83.4	60.2
3	267.0	-13.4	119	7.87	2.98	2.83	3.95	5.31	75.4	53.3
4	246.2	-2.1	125	7.50	2.62	2.79	3.42	5.08	76.5	54.9
5	368.4	2.8	128	7.63	3.83	3.86	4.95	6.63	77.4	58.2
6	348.4	12.4	113	4.81	4.10	4.25	5.66	8.67	72.5	49.0
7	352.7	20.8	104	4.58	4.51	4.78	6.22	8.90	72.5	53.7
8	335.6	45.1	100	4.72	4.46	5.06	5.81	8.83	76.7	57.3
9	287.1	42.7	102	5.47	3.74	4.30	4.63	7.29	80.7	59.0
10	331.3	40.6	113	4.67	3.90	4.38	5.10	7.92	76.4	55.3
11	333.5	30.1	121	4.88	3.67	4.00	4.75	7.16	77.3	55.9
12	423.4	41.9	136	4.30	4.14	4.55	5.14	7.61	80.5	59.8
13	610.4	80.8	135	2.77	6.01	6.81	9.20	13.07	65.3	52.1
14	723.3	102.8	132	2.45	7.29	8.32	10.98	15.35	66.4	54.2
15	676.8	103.0	139	2.50	6.48	7.46	10.82	14.89	59.9	50.1
16	1,230.9	83.9	143	2.55	11.45	12.23	15.86	19.63	72.2	62.3
17	991.8	76.6	153	2.64	8.69	9.29	12.59	15.88	69.0	58.5
18	1,091.0	45.5	166	2.78	8.74	9.11	11.67	14.37	74.9	63.4
19	1,118.8	74.1	166	2.60	8.96	9.56	12.43	15.44	72.1	61.9
				System 2						
1	86.4	6.5	140	26.21	0.82	0.88	0.98	1.44	83.5	61.2
2	75.7	-9.1	150	26.89	0.67	0.59	0.82	1.17	82.2	50.6
3	80.7	5.3	179	28.11	0.60	0.64	0.71	0.99	84.9	64.9
4	79.3	7.0	180	23.64	0.59	0.64	0.68	1.00	86.8	64.2
5	105.7	14.9	177	18.08	0.79	0.91	0.87	1.39	90.8	65.7
6	120.0	9.9	175	16.65	0.91	0.99	1.18	1.66	77.1	59.5
7	138.5	19.6	157	20.83	1.17	1.34	1.39	1.91	84.3	70.3
8	151.3	8.5	163	21.82	1.23	1.30	1.46	1.88	84.1	69.3
9	154.2	3.9	173	18.45	1.19	1.22	1.37	1.81	86.7	67.4
10	159.2	3.8	169	17.88	1.25	1.28	1.46	1.95	85.8	65.5
11	156.4	-0.7	156	19.74	1.33	1.33	1.56	2.04	85.2	65.1
12	162.1	5.6	151	19.55	1.43	1.48	1.67	2.12	85.7	69.9
13	267.8	7.6	150	12.83	2.37	2.44	2.79	3.47	85.0	70.4
14	177.8	18.0	134	12.71	1.76	1.94	2.00	2.86	88.0	67.9
15	167.1	19.6	129	15.76	1.72	1.92	1.98	2.74	86.7	70.0
16	172.0	2.1	137	9.73	1.67	1.69	1.95	2.84	85.7	59.5
17	185.6	11.7	147	13.14	1.68	1.79	1.98	2.71	84.7	66.0
18	219.9	16.4	155	11.83	1.89	2.03	2.11	2.88	89.5	70.5
19	226.3	15.4	157	16.79	1.92	2.05	2.31	2.87	83.0	71.4
20	456.2	40.4	147	3.93	4.13	4.49	6.76	9.07	61.1	49.5
21	880.4	77.4	140	3.13	8.36	9.10	9.87	13.21	84.7	68.9
22	759.0	86.9	150	3.16	6.73	7.50	7.79	10.95	86.4	68.5
23	760.4	81.0	161	3.26	6.28	6.95	7.25	10.03	86.6	69.3
24	866.1	38.1	165	3.36	6.98	7.29	7.88	10.25	88.6	71.1

[a] X_1 = reactor sample VSS, mg/ℓ.

[b] θ_c = cell residence time, days.

[c] q_{H_2} = mg H_2-COD removed/mg VSS - day.

[d] q_T = mg total (H_2 plus soluble) COD removed/mg VSS - day.

[e] $(L_c)_{H_2}$ = mg H_2-COD added/mg VSS-day.

[f] $(L_c)_T$ = mg total COD added/mg VSS-day.

[g] E_{H_2} = efficiency of H_2-COD removal, $100 \frac{q_{H_2}}{(L_c)_{H_2}}$

[h] E_T = efficiency of total COD removal, $100 \frac{q_T}{(L_c)_T}$

As shown in Table VIII, the enrichment cultures in Systems 1 and 2 operated with mean cell residence times of 2.5 to 28 days, and hydrogen COD rates of 0.6 to 11.5 mg H_2-COD removed per mg VSS-day.

Determination of Kinetic Constants

The kinetic characteristics q_{H_2}, $\frac{10}{q_{H_2}}$, q_T, $\frac{10}{q_T}$, $\frac{1{,}000}{(S_1)_G}$, and $\frac{1}{\theta_c}$ were calculated from the results of Table VIII and are summarized in Table IX. Data in seven groups of three or more consecutive balance periods fulfilled steady state criteria:

System 1: BP (Balance period) 1 to 4, 6 to 8, and 17 to 19.

System 2: BP 2 to 4, 9 to 11, 16 to 18, and 22 to 24.

In all but three of the balance periods between steady state intervals, the hydrogen removal rate coefficient (q_{H_2}) and the hydrogen substrate concentration $(S_1)_G$ fluctuated greater than ± 10 percent. In BP 9 to 11 (System 1) the steady state was not achieved because the cell residence time θ_c did not remain constant.

The kinetic constants Y, k_d, $\hat{q}$, and K_s were determined for both H_2-COD and total COD removal rate coefficients, using the steady state data and the graphical methods shown in Figures 4 and 5. The steady state data are shown in Figures 13 to 16 and the equations of best fit were determined by the method of least squares. The kinetic constants for H_2-COD and the total COD removal are summarized in Table X.

TABLE IX

GROWTH AND KINETIC CHARACTERISTICS - ENRICHMENT STUDY

Balance Period	System 1							System 2								
	q_{H_2} [b]	$\frac{10}{q_{H_2}}$	q_T [c]	$\frac{10}{q_T}$	$(S_1)_G$ [d]	$\frac{1000}{(S_1)_G}$	$\frac{1}{\theta_c}$ [e]	Balance Period	q_{H_2}	$\frac{10}{q_{H_2}}$	q_T	$\frac{10}{q_T}$	$(S_1)_G$	$\frac{1000}{(S_1)_G}$	$\frac{1}{\theta_c}$
1 [a]	3.18	3.14	3.32	3.01	97.8	10.22	0.1313	1	0.82	12.15	0.88	11.30	21.6	46.29	0.0381
2 [a]	3.28	3.05	3.41	2.93	96.5	10.36	0.1425	2 [a]	0.67	14.86	0.59	16.89	15.5	64.52	0.0372
3 [a]	2.98	3.36	2.83	3.53	102.8	9.73	0.1270	3 [a]	0.60	16.64	0.64	15.61	14.8	67.58	0.0356
4 [a]	2.62	3.82	2.79	3.58	86.5	11.56	0.1333	4 [a]	0.59	17.02	0.64	15.64	14.3	69.93	0.0423
5	3.83	2.61	3.86	2.59	91.5	10.93	0.1311	5	0.79	12.56	0.91	11.01	13.8	72.46	0.0553
6 [a]	4.10	2.44	4.25	2.35	124.5	8.03	0.2077	6	0.91	10.94	0.99	10.10	17.9	55.87	0.0601
7 [a]	4.51	2.22	4.78	2.09	121.3	8.24	0.2186	7	1.17	8.50	1.34	7.45	35.6	28.09	0.0480
8 [a]	4.46	2.24	5.06	1.98	115.1	8.69	0.2119	8	1.23	8.08	1.30	7.65	39.0	25.64	0.0458
9	3.74	2.67	4.30	2.33	125.6	7.96	0.1827	9 [a]	1.19	8.41	1.22	8.21	28.6	34.97	0.0542
10	3.90	2.56	4.38	2.28	137.8	7.26	0.2141	10 [a]	1.25	7.96	1.28	7.78	28.2	35.46	0.0559
11	3.67	2.72	4.00	2.50	125.6	7.96	0.2050	11 [a]	1.33	7.48	1.33	7.52	29.9	33.44	0.0507
12	4.14	2.42	4.55	2.20	130.4	7.67	0.2328	12	1.43	6.99	1.48	6.75	28.5	35.09	0.0512
13	6.01	1.66	6.81	1.47	202.4	4.95	0.3611	13	2.37	4.20	2.44	4.08	40.1	24.94	0.0779
14	7.29	1.37	8.32	1.20	300.1	3.33	0.4085	14	1.76	5.65	1.94	5.13	44.7	22.37	0.0787
15	6.48	1.54	7.46	1.34	374.5	2.67	0.4007	15	1.72	5.79	1.92	5.18	35.3	28.33	0.0635
16	11.45	0.87	12.23	0.82	405.3	2.47	0.3925	16 [a]	1.67	5.97	1.69	5.90	38.1	26.24	0.0913
17 [a]	8.69	1.15	9.29	1.08	406.1	2.46	0.3781	17 [a]	1.68	5.94	1.79	5.58	41.9	23.87	0.0761
18 [a]	8.74	1.14	9.11	1.10	390.9	2.56	0.3602	18 [a]	1.89	5.29	2.03	4.92	41.6	24.04	0.0845
19 [a]	8.96	1.12	9.56	1.05	372.3	2.69	0.3843	19	1.92	5.21	2.05	4.88	46.8	21.37	0.0595
								20	4.13	2.42	4.49	2.22	141.7	7.06	0.2545
								21	8.36	1.19	9.10	1.10	220.3	4.54	0.3198
								22 [a]	6.73	1.48	7.50	1.33	198.9	5.03	0.3163
								23 [a]	6.28	1.59	6.95	1.43	199.1	5.02	0.3063
								24 [a]	6.98	1.43	7.29	1.37	195.3	5.12	0.2974

[a] Balance periods at which steady-state criteria were fulfilled.

[b] q_{H_2} = mg H_2-COD removed/mg VSS - day.

[c] q_T = mg total COD removed/mg VSS - day.

[d] $(S_1)_G$ = partial pressure of H_2 in reactor, mm Hg.

[e] θ_c = cell residence time, days.

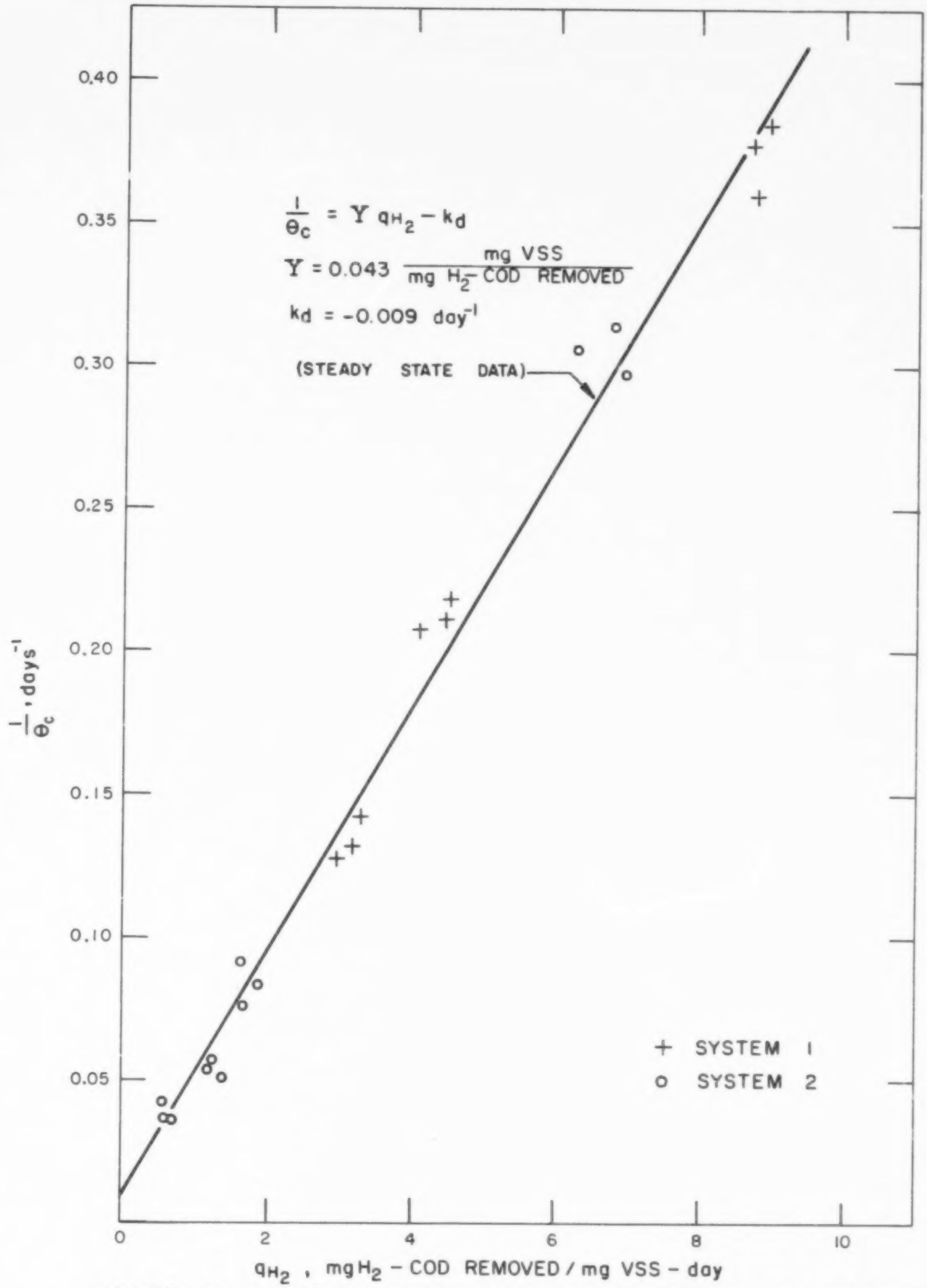


FIGURE 13. DETERMINATION OF YIELD CONSTANT AND SPECIFIC ORGANISM DECAY RATE – HYDROGEN COD REMOVAL BASIS

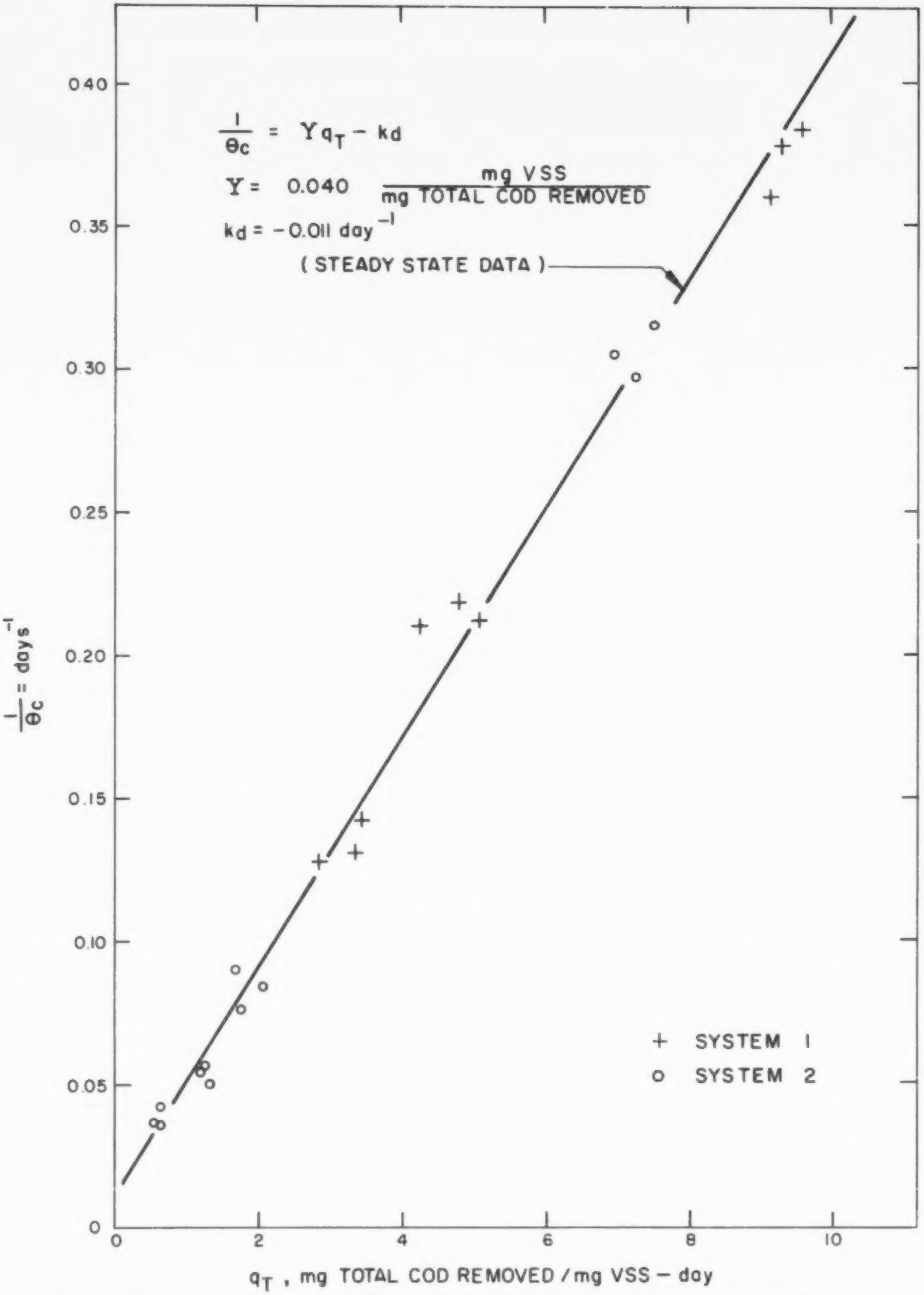


FIGURE 14. DETERMINATION OF YIELD CONSTANT AND SPECIFIC ORGANISM DECAY RATE - TOTAL COD REMOVAL BASIS

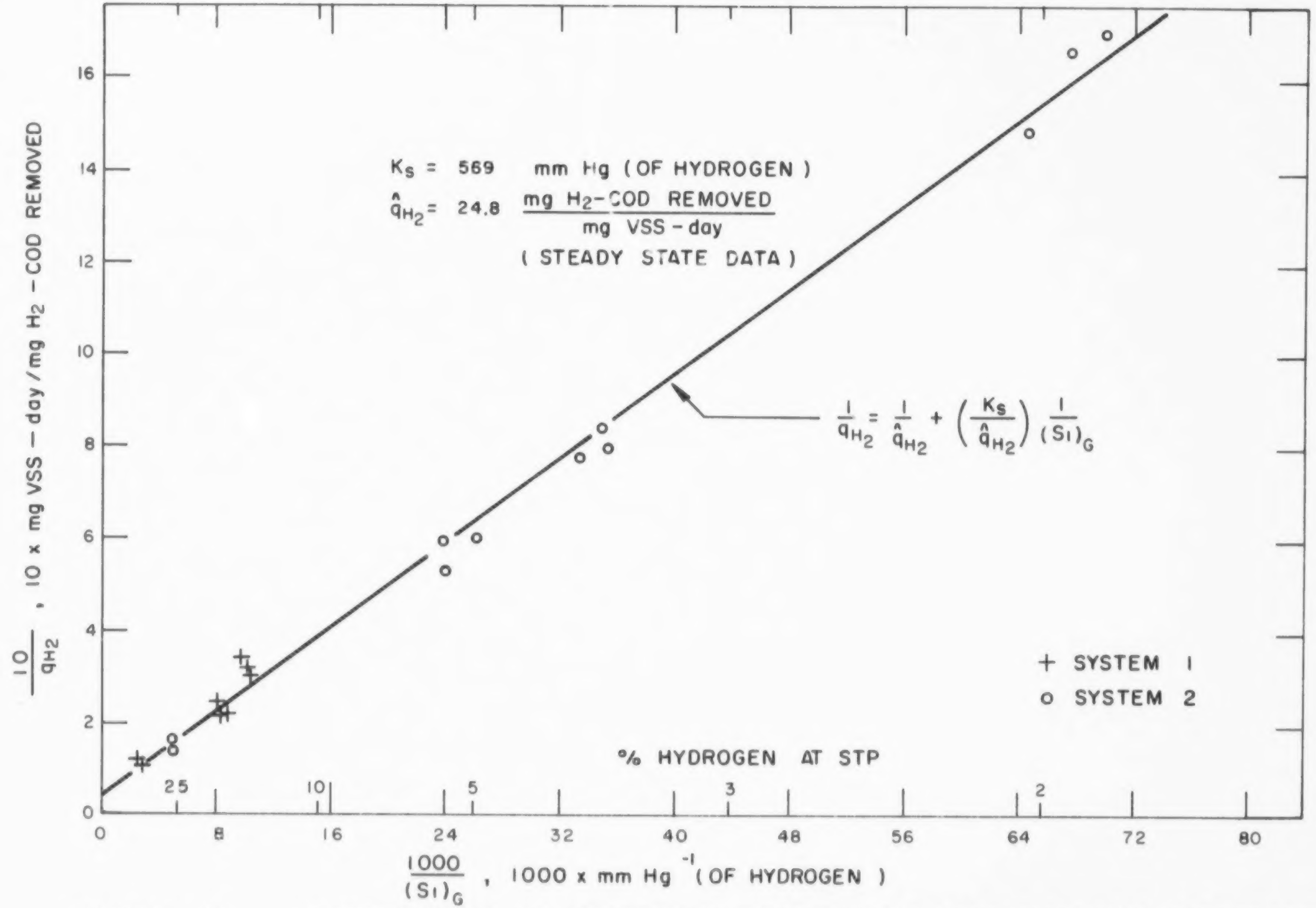


FIGURE 15. DETERMINATION OF SATURATION CONSTANT (K_S) AND MAXIMUM SUBSTRATE METABOLIC COEFFICIENT ($\hat{q}_{H_2}$) - HYDROGEN COD REMOVAL BASIS

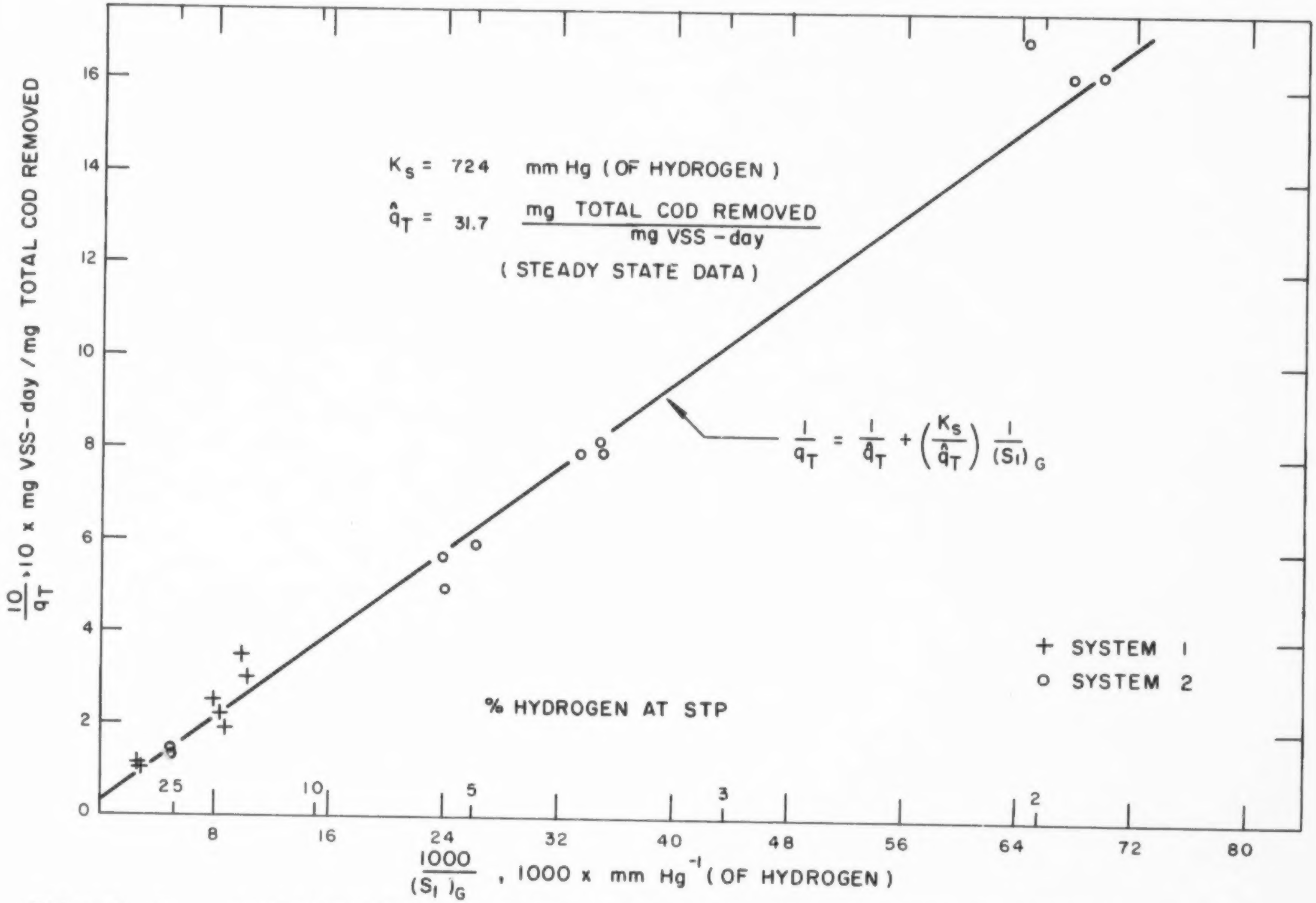


FIGURE 16. DETERMINATION OF SATURATION CONSTANT (K_s) AND MAXIMUM SUBSTRATE METABOLIC COEFFICIENT ($\hat{q}_T$) - TOTAL COD REMOVAL BASIS

TABLE X

ENRICHMENT GROWTH AND KINETIC CONSTANTS

Constants	Units	COD Removal Basis	
		Hydrogen	Total
Y	mg VSS per mg COD Removed	0.043	0.040
k_d	Days^{-1}	- 0.009	- 0.011
$\hat{q}$	mg COD Removed per mg VSS-Day	24.8	31.7
K_S	Hydrogen, mm Hg	569.	724.
$\hat{\mu}$[a]	Days^{-1}	1.06	1.26
g[b]	Hours	15.6	13.2

[a] $\hat{\mu} = Y\hat{q}$ (Equation 8).

[b] $g = \frac{\ln 2}{\hat{\mu}}$ (Equation 10).

The maximum growth rates of the enrichment culture were calculated from the kinetic constants of Table X using Equation 18:

$$\hat{\mu} = 1.06 \text{ days}^{-1} \text{ (hydrogen COD data)}$$

$$\hat{\mu} = 1.26 \text{ days}^{-1} \text{ (total COD data)}$$

The generation times of the enrichment culture were calculated from the maximum growth rates by Equation 10:

$$g = 15.6 \text{ hours (hydrogen COD data)}$$

$$g = 13.2 \text{ hours (total COD data)}$$

Figure 17 shows a plot of substrate removal rate coefficient (q_{H_2}) versus partial pressure of hydrogen $(S_1)_G$, with the equation of best fit indicated. It is apparent that the experimental data fit the Michaelis-Menten model satisfactorily, and that hydrogen as measured by $(S_1)_G$ was the growth limiting nutrient in the enrichment fermenters.

The maximum experimentally measured rates of hydrogen gas removal and methane gas production are reported in Table XI. The maximum measured hydrogen removal rate coefficient q_{H_2} was 11.45 mg H_2-COD per mg VSS-day; the maximum measured methane production rate q_{CH_4} was 9.03 mg CH_4-COD per mg VSS-day. Both data were obtained in System 1 at BP (Balance Period) 16.

TABLE XI

MAXIMUM MEASURED GAS TRANSFER RATES

Symbol	Units	Maximum Rate[a]	
		H_2	CH_4
q	mg Gas COD Transferred / mg VSS-Day	11.45	9.03
q	mℓ Gas Transferred / mg VSS-Day	16.04	3.16
qX_1	mℓ Gas Transferred / mℓ Reactor Volume-Day	2.30	0.50[b]

[a] Date of System 1--Balance Period 16, unless noted.

[b] System 1--Balance Period 19.

Statietical Evaluation of Results

The enrichment cultures were developed from the same inoculum biomass. However, there existed a possibility that different microbial populations may have developed in the two reactors. For this reason,

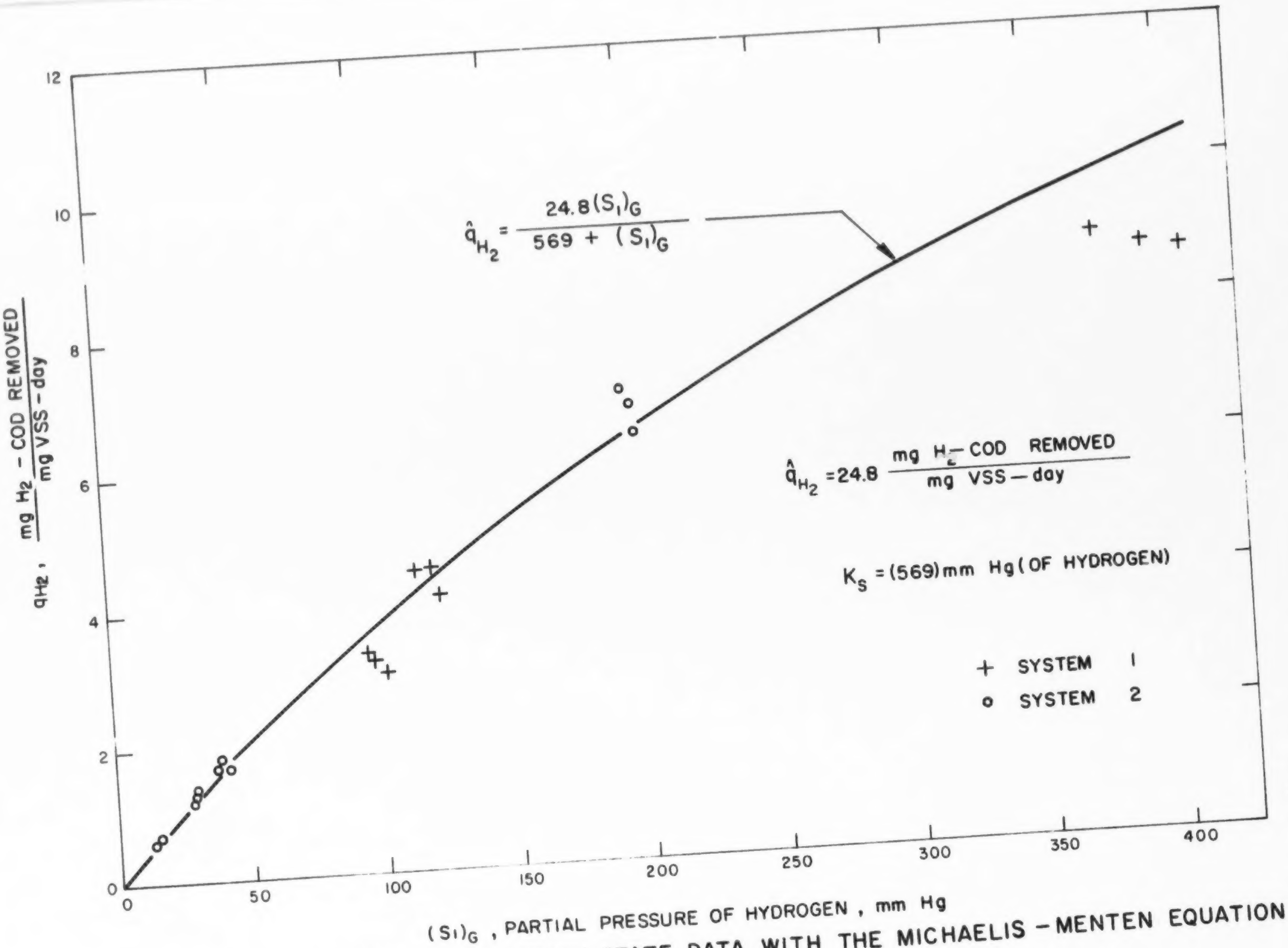


FIGURE 17. COMPARISON OF STEADY STATE DATA WITH THE MICHAELIS – MENTEN EQUATION OF BEST FIT – HYDROGEN COD REMOVAL BASIS

statistical analyses of the performance of the two cultures were undertaken.

The performance of the enrichment cultures was assessed using the following COD material balance indices:

1. Ratio of CH_4-COD produced and H_2-COD removed.
2. Ratio of CH_4-COD produced and total (H_2plus soluble) COD removed.
3. Ratio of cell COD produced and H_2-COD removed.
4. Ratio of cell COD produced and total COD removed.
5. Ratio of H_2-COD removed and total COD removed.
6. COD recovery: ratio of sum of CH_4-COD and cell COD produced to total COD removed

The mean values and standard deviations from the mean of each index are reported by System in Table XII, and by both cell residence time (θ_c) and System in Table XIII. Data from all balance periods reported in Table VIII were used to compute the indices.

COD Material Balance --The average COD recovery (ratio of sum of CH_4-COD and cell COD produced to total assimilated COD) was 94.9 percent in System 1, and 93.3 percent in System 2, as reported in Table XII. Hydrogen COD comprised 93 percent of the total COD uptake in System 1, and 94.3 percent in System 2; the balance of the total COD removed in each system was due to soluble COD uptake. Methane accounted for about 93 percent of the product COD (sum of cell COD and CH_4-COD) in both Systems 1 and 2. These are reasonable COD balances and indicate that the major elements of the COD balance

TABLE XII

STATISTICAL ANALYSIS OF COD BALANCE INDICES[a]

Index	System 1		System 2		t
	$\bar{n}$	σ	$\bar{n}$	σ	
COD Recovery, %	94.9	7.2	93.3	9.9	0.58[b]
$\frac{CH_4\text{-COD Produced}}{H_2\text{-COD Removed}}$, %	91.2	12.0	88.3	13.4	0.71[b]
$\frac{CH_4\text{-COD Produced}}{\text{Total COD Removed}}$, %	85.3	12.4	84.0	14.7	0.30[b]
$\frac{\text{Cell COD Produced}}{H_2\text{-COD Removed}}$, %	6.8	1.1	6.7	1.6	0.22[b]
$\frac{\text{Cell COD Produced}}{\text{Total COD Removed}}$, %	6.3	0.9	6.3	1.6	0.00[b]
$\frac{H_2\text{-COD Removed}}{\text{Total COD Removed}}$, %	93.0	4.7	94.3	5.4	0.81[b]

[a] All balance periods included.

[b] Insignificant at 95 per cent confidence level.

NOTE: $\bar{n}$ = mean value.

σ = standard deviation.

t = "t" test of significance between two sample means.

TABLE XIII

VARIATION OF COD BALANCE INDICES WITH CELL RESIDENCE TIME

System	Balance Period Group	Cell Residence Time Days[a]	COD Recovery %[a]	COD Produced/COD Removed, %[a]				$\frac{H_2\text{-COD Removed}}{\text{Total COD Removed}}$, %[a]	Acetic Acid COD, Per cent of Effluent Soluble COD
				$\frac{CH_4}{H_2}$	$\frac{CH_4}{\text{Total}}$	$\frac{\text{Cell}}{H_2}$	$\frac{\text{Cell}}{\text{Total}}$		
2	1- 4	26	98.8	92.4	91.2	8.2	8.0	98.1	-
2	5-12	19	88.8	81.4	77.2	6.6	6.2	94.3	18.4[b]
2	13-19	13	95.9	94.7	88.9	6.0	5.6	93.9	11.0[c]
1	1- 5	7.5	95.6	88.7	87.2	6.1	5.9	98.1	28.5[b]
1	6-12	4.8	95.5	93.9	85.7	7.3	6.7	91.2	20.0[c]
2	20-24	3.4	92.3	89.7	82.4	6.7	6.2	91.9	10.8[b]
1	13-19	2.6	93.9	91.4	83.4	6.9	6.2	91.3	13.9[c]

[a] Mean value of balance period group.

[b] Single observation only.

[c] Mean value.

were included. The COD recoveries were consistent for both systems. The above data indicate that the corrections used for diffusion losses were reasonable.

The COD balance indices are grouped by θ_c (cell residence time) and by System in Table XIII to permit assessment of the effects of θ_c on the indices. The only index which shows significant correlation with θ_c is the ratio of H_2-COD removed to total COD removed, which averages about 96 percent at $\theta_c > 7.5$ days and 91 percent at $\theta_c < 4.8$ days. Because of the transition in this index, it appears that the average soluble COD uptake increased in both systems from four percent of the total COD removal at $\theta_c > 7.5$ days to nine percent at $\theta_c < 4.8$ days. However, the ratios of CH_4-COD produced to (1) H_2-COD removed and (2) total COD removed showed only random variation with θ_c, and it can be concluded that the percent conversion of assimilated hydrogen and soluble COD to methane showed no significant correlation with cell residence time.

Similarity of Enrichment Systems.-- Each COD balance index for Systems 1 and 2 was evaluated by the "t" test and the results are reported in Table XII. None of the "t" values were significant at the 95 percent confidence level; thus it can be concluded that the performance of the two systems was essentially identical at a high confidence level and that the data of the two systems can be combined for kinetic analysis.

Volatile Acids.--The volatile acid characteristics of the two systems are summarized in Table XXIV of Appendix E. Acetic acid constitutes essentially the total volatile acids because only trace quantities of the other acids were detected with the chromatograph.

The ratios of acetic acid COD to effluent soluble COD for both systems are reported in Table XIII. The acetic acid COD ranged between 10.8 percent and 28.5 percent of the effluent soluble COD, and the percent ratio did not correlate either with system or with θ_c. Moreover, the ratio did not correlate with the increase in the average ratio of soluble COD removed: total COD removed.

Discussion

Kinetic Evaluation of Enrichment Cultures

Enrichment cultures were used in this study because a pure culture of the hydrogen assimilating methane bacterium was not available. Consequently, the data may represent the activity of several bacterial groups in addition to hydrogen assimilation by the methane bacterium.

Of major concern is the possible production of acetic acid from hydrogen and carbon dioxide by contaminant organisms in the enrichment culture. Such a source of acetic acid may not be the only one, as acetic acid is also a decomposition product of the yeast extract and mercaptoacetic acid constituents of the liquid substrate (Table III). About 10 mg/l of acetic acid was present in the liquid substrate (Table IV). It appears, regardless of source, that acetic acid found in the enrichment systems may have supported an undetermined amount of methane production during the study.

The principal advantage in the use of kinetic characteristics such as the maximum hydrogen removal rate coefficient ($\hat{q}_{H_2}$), generation time (g), and maximum growth rate ($\hat{\mu}$) is to permit comparison of the results of this study and those of other biological systems, particularly methanogenic fermentations.

Generation Time.--The generation times of the bacteria in the hydrogen enrichment cultures were found to be:

g = 15.6 hours (hydrogen COD data)
g = 13.2 hours (total COD data)

Generation times for enrichment cultures of methane bacteria reported by McCarty [57], Speece and McCarty [58], and Lawrence and McCarty [59] or estimated from their data are summarized in Table XIV. Minimum generation time data for methane bacteria are summarized from Table XIV as follows:

Methanol substrate: g = 1.4 days
Formate substrate: g = 2.1 days
Acetate substrate: g = 2.2 days
Propionate substrate: g = 2.2 days
Butyrate substrate: g = 1.9 days

It is apparent that the generation times of the hydrogen enrichment organisms are about one-third to one-fourth those described above from the minimum generation times reported by McCarty *et al* [57] [58] [59] and others. The generation times of the hydrogen assimilating methane bacterium appear to be the minimum generation times reported for methanogenic bacteria grown on any type of substrate in enrichment culture.

TABLE XIV

GENERATION TIMES AND YIELD COEFFICIENTS OF METHANE BACTERIA

Substrate Fermented	Minimum Residence Time (θ_w) days	Generation Time (g) days	Reference
Methanol	2	1.4[a]	McCarty [57]
Formate	3	2.1[a]	McCarty [57]
Acetate	5	3.5[a]	Speece and McCarty [58]
Acetate	3.1	2.2	Lawrence and McCarty [59]
Propionate	7.5	5.2[a]	McCarty [57]
Propionate	3.2	2.2	Lawrence and McCarty [59]
Butyrate	2.7	1.9	Lawrence and McCarty [59]

[a] Estimated from: $g \simeq (\ln 2)\, \theta_w$ (Equation 29).

Andrews et al [40] have reported methane production at a hydraulic residence time of 0.75 days, which is equivalent to a generation time of 12.5 hours, but it is believed that adhered wall growths of methane bacteria may have influenced the results of Andrew's study. Both hydrogen and significant concentrations of volatile acids (2,540 mg/l as acetic acid) were present at the residence time of 0.75 days.

The minimum generation time of the acetate-utilizing methane bacteria is 2.2 days, which is equivalent to a critical cell residence time (θ_w) of 3.1 days as reported by Lawrence and McCarty [58] and shown in Table XIV. Thus in the absence of adhered wall growths, the acetate-utilizing methane bacteria would be expected to wash out of a continuous flow reactor at a cell residence time less than 3.1 days. The minimum cell residence time at which the hydrogen enrichment cultures were studied was 2.5 days, which is about 20 percent less than the critical cell residence time of 3.1 days for acetate-assimilating methane bacteria. Because adhered wall growths were removed daily from the reactor walls of the enrichment fermenters, it is expected that acetate-utilizing methane bacteria would have washed out of the enrichment fermenters at the lowest cell residence times (2.5 days) employed.

Substrate Removal Rate Coefficients.--The maximum substrate removal rate coefficients of the enrichment systems were reported in Table X as follows:

$$\hat{q}_{H_2} = 24.8 \text{ mg } H_2\text{-COD removed/mg VSS-day}$$

$$\hat{q}_T = 31.7 \text{ mg total COD removed/mg VSS-day}$$

Substrate removal rate coefficients, saturation constants, yield coefficients, and organism decay rates for several anaerobic and aerobic systems are reported in Table XV. As summarized by Pearson [41] and in this study, the kinetic constants have been reported by the authors cited in Table XV or computed from their data. Variation of substrates, analytical techniques, and the unique character of each system makes objective comparison difficult, but Table XV does permit comparison of the substrate removal rate constants and process coefficient obtained in this study with those reported previously for anerobic and aerobic processes.

The maximum substrate removal rates reported by Lawrence and McCarty [59] shown in Table XV can be compared with the results of this study. It is of interest to note that the maximum substrate removal rate coefficients of this study (24.8 mg H_2-COD removed/mg VSS-day; and 31.7 mg Total COD removed/mg VSS-day) are three to four times greater than the maximum rates of 8 to 9 mg COD removed/mg VSS-day for acetic, propionic, and butyric acid as reported by Lawrence and McCarty [59]. It appears that the hydrogen assimilating methane bacterium is capable of three or four times greater maximum energy turnover (as measured by $\hat{q}$) than are the methane bacteria using acetic, propionic, or butyric acids.

The maximum substrate assimilation rates of this study exceed those of Stewart [39] (0.37 mg COD removed/mg VSS-day) and Agardy et al [38] (0.79 mg COD removed/mg VSS-day) by 30 to 50 times. Stewart and Agardy et al used a similar dextrose, peptone and beef extract, substrate which supported the growth of acid-producing organisms in

TABLE XV

TYPICAL BIOLOGICAL PROCESS CONSTANTS

Process	Substrate	$\hat{q}^a$		K_s mg/ℓ		Y^b		k_d day^{-1}	Reference
		BOD	COD	BOD	COD	BOD	COD		
	Settled Sewage (laboratory)					0.50		0.055	Heukelekian *et al.* [60]
	Skim Milk (laboratory)	5.1		100		0.48		0.045	Gram [61]
Aerobic	Glucose (laboratory)	3.0		355		0.42		0.087	Stack and Conway [62]
Activated Sludge	Settled Sewage (field)						0.45	0.05	Pearson and Haas [63]
	Synthetic Sewage (laboratory)						0.315	0.036	Eckhoff and Jenkins [64]
	Settled Sewage (pilot plant)		48.5				0.44	0.14	Jenkins and Menar [65]
	Dextrose, Peptone, Beef Extract		0.37		5,700		0.18	0.025	Stewart [39]
	Dextrose, Peptone, Beef Extract		0.79		13,000		0.104	0.03	Agardy *et al.* [[illegible]9]
Anaerobic	Glucose and Starch						0.46	0.088	Speece and McCarty [58]
Continuous Flow Reactor	Amino and Fatty Acids						0.054	0.038	Speece and McCarty [58]
(Laboratory Methane Fermentations)	Nutrient Broth						0.076	0.014	Speece and McCarty [58]
	Acetic Acid (enriched culture)		8.7		154		0.041	0.019	Lawrence and McCarty [59]
	Propionic Acid (enriched culture)		7.7		40		0.048	0.010	Lawrence and McCarty [59]
	Butyric Acid (enriched culture)		8.3		37		0.047	0.027	Lawrence and McCarty [59]
	This Study (Hydrogen COD data) (enriched culture)		74.8		549^c		0.043	-0.009	
	This Study (Total COD data)		51.7		704^c		0.040	-0.011	

[a] $\hat{q}$ = mg BOD or COD removed/mg VSS-day. [b] Y = mg VSS/mg BOD or COD removed. [c] Hydrogen, mm Hg.

addition to methanogenic bacteria. The constants of Stewart and Agardy et al are based on a mixed population of acid forming and methanogenic bacteria and a markedly different substrate rather than on enriched cultures of essentially methanogenic hydrogen-utilizing bacteria.

Jenkins [65] reported a maximum substrate utilization rate of 42.5 mg COD removed per mg VSS-day in a study on dispersed growths of aerobic organisms fed a settled sewage substrate. The data of Jenkins indicate a high substrate removal rate capacity of dispersed growth aerobic systems which exceeded by several times the maximum rates of 5.1 mg BOD (not COD) removed/mg VSS-day reported by Gram [61] Stack and Conway [62] for glucose. The maximum rate of 42.5 mg COD removed/mg VSS-day reported by Jenkins for a dispersed growth aerobic system is 30 to 50 percent larger than the maximum substrate removal rate coefficients of this study (24.8 mg H_2-COD removed/mg VSS-day; 31.7 mg total COD removed/mg VSS-day).

The maximum hydrogen removal rate calculated from the batch pure culture data of Barker [24] for M. omelianskii was 12 mg H_2-COD/mg VSS-day. Although the effects of batch culture conditions on the maximum rate of hydrogen removal in Barker's study are unknown, it appears that the maximum hydrogen assimilation rate of the enrichment culture systems of this study was more than twice that of the pure culture investigated by Barker.

Saturation Constant.--The saturation constants of the enrichment systems were reported in Table X as follows:

K_s = 569 mm Hg of hydrogen, or 75 percent hydrogen atmosphere (based on hydrogen COD data).

K_s = 724 mm Hg of hydrogen, or 95 percent hydrogen atmosphere (based on total COD data).

Under normal conditions of anerobic fermentation of sewage sludges, hydrogen comprises generally less than three percent of the digester gas (Burgess and Wood [26]). Thus it appears that the substrate removal rate coefficient of the hydrogen assimilating methane bacterium in a normal sewage sludge fermentation is less than about three percent of the maximum hydrogen removal rate coefficient, as estimated from the Michaelis-Menten model (Equation 6).

No data were found which can be compared directly with the saturation constants of this study. Saturation constants of 13,000 mg COD/l (Agardy et al [38]) and 5,700 mg COD/l (Steward [39]) were obtained in studies on laboratory anaerobic systems using dextrose, peptone, and beef extract substrates. The saturation constants of Agardy et al [38] and Stewart [39] were determined using a gross COD measurement which includes refractory organic and degradable organic material rather than measuring just the concentration of a specific rate-limiting substrate. The saturation constants reported by Lawrence and McCarty [59] for acetic acid (154 mg acetic acid COD/l), propionic acid (40 mg propionic acid COD/l, and butyric acid (7 mg butyric acid COD/l) were based on the specific rate-limiting substrate, and are more comparable to the aerobic saturation constants of 100 mg BOD/l reported by Gram [61] for skim milk, and 355 mg BOD/l reported by Stack and Conway [62] for glucose; than to the data of Agardy et al and Stewart. Because the saturation constant is dependent on the method of analysis of substrate concentration, direct comparison of the anaerobic and aerobic systems on the basis of the saturation constants reported in Table XV is not possible. If saturation constants are determined on the basis of gross COD concentrations rather than on concentrations of a specific rate-limiting compound, then the saturation constants of anaerobic fermentations of complex substrates (such as sewage sludges) are larger than in aerobic systems; thus aerobic systems would be more suited to the treatment of dilute organic wastes whereas anaerobic systems are better suited for concentrated waste streams.

Yield Coefficient.--The yield coefficients (Y) of cells produced in the hydrogen enrichment systems were reported in Table X as follows:

Y = 0.043 mg VSS/mg H_2-COD removed

Y = 0.040 mg VSS/mg total COD removed

The yields reported by Lawrence and McCarty [59] for enriched culture fermentations of three volatile acids were reported in Table XV as follows:

Y = 0.041 mg VSS/mg COD removed (acetic acid)

Y = 0.042 mg VSS-mg COD removed (propionic acid)

Y = 0.047 mg VSS/mg COD removed (butyric acid)

Thus the yield coefficients for the hydrogen assimilating methanogenic bacterium determined in this study are essentially the same as the yield coefficients reported by Lawrence and McCarty for volatile acid utilizing methane bacteria (all data ranging between 4.0

and 4.7 percent) and show that only about four to five percent the substrate COD utilized in methane fermentations is synthesized into methanogenic bacterial cells.

Agardy et al [38] and Stewart [39] measured yields of 0.104 and 0.18 mg VSS/mg COD removed respectively in anerobic fermentations of complex (dextrose, peptone, and beef extract) substrates, as reported in Table XV. The yield coefficients obtained from studies on aerobic systems and reported in Table XV ranged from 0.3 to 0.5 mg VSS per mg BOD or COD. Thus the yield coefficients of the cited aerobic systems appear to be generally larger than those of anaerobic systems, and the yield coefficients of four to five percent obtained in the enriched culture methane fermentations are less than those normally obserted in anerobic fermentations of complex substrates.

Organism Decay Term.--The organism decay or material balance terms k_d for this study were reported in Table X as below:

$$k_d = -0.009 \text{ days}^{-1} \text{ (hydrogen COD data)}$$

$$k_d = -0.011 \text{ days}^{-1} \text{ (total COD data)}$$

The negative values of k_d obtained in this study are contrary both to theory and to the results of other kinetic studies. The organism decay term, k_d, is a material balance correction to the growth rate μ to account for endogenous breakdown of cell material, cell death and lysis, and the resynthesis of cell matter from the lysis products of other cells. The first three factors increase the decay term, whereas the resynthesis of cells decreases the decay term. The magnitude of resynthesis cannot be determined by present methodology (1967), but resynthesis is limited by the energy in the lysis products available for cell systhesis. Resynthesis cannot equal cell losses by lysis because of the inefficiency of biological energy transfer; thus resynthesis cannot account for the negative decay constants.

A change in the viable fraction of organisms due to changing growth rates would appear to be the major cause of the negative decay constants. Postgate and Hunter [42] have shown that loss of organisms by death can be significant at low growth rates. Their work was done with continuous cultures of Aerobacter aerogenes using carbon, nitrogen, phosphorus, and sulfur as the growth-limiting nutrients. Steady states were obtained in which constant proportions of the organisms were dead and as the cell residence time was increased the percentage of viable organisms decreased. For example, with glycerol as the limiting nutrient 96.6 percent of the organisms were viable at a residence time of 4.31 hours, 86.1 percent at a residence time of 33.9 hours, and 37.8 percent were viable at a residence time of 263 hours. It appears that the percentage viable organisms decreased nearly 50 percent as the cell residence time increased by a factor of about eight (33.9 hours to 263 hours).

VSS (volatile suspended solids) is the measure of the viable and nonviable organisms which is used in this study to compute the substrate removal rate coefficient. Because the substrate removal rate at each growth rate was computed using VSS its value is less than the substrate removal rate which would be obtained at the same growth rate using a denominator term based upon only viable organisms.

The difference between the two rates should increase if the percentage of viable organisms decreased with decreasing growth rates. If the percentage of viable organisms decreased with decreasing growth rates, the ordinate intercept ($-k_d$) of the best fit cell continuity equation (Equation 7) would be greater using substrate removal rates on a VSS basis than the ordinate intercept obtained with substrate removal rates based on viable organisms only. A greater ordinate intercept of the cell continuity equation,

$$\frac{1}{\theta_c} = Yq - k_d, \tag{7}$$

is analogous to a reduction of the decay constant k_d (Figure 4), and it is apparent that a sufficient reduction in the viable portion of the organism measure at low growth rates can reduce the decay constant k_d from a positive to a negative value.

No attempts were made to determine the viable fraction of the VSS in this study; instead, the viable fraction was assumed to be a constant portion of the VSS. The enrichment cultures were operated over a range of about one order of magnitude (2.5 to 28 days) in cell residence times. This range in residence time might be sufficient to cause more than a 50 percent change in the percentage of viable organisms based upon the findings of Postgate and Hunter [42] cited previously. The cell residence time variation in this study may have caused a significant change in percent viable organisms which could not be detected with the VSS parameter, and which may have reduced the decay constants to the negative values observed.

Methane Production Rates.--The highest observed rate of methane production in the hydrogen enrichment systems was 9.03 mg CH_4-COD produced/mg VSS-day (Table XI). The maximum rates of conversion of volatile acids to methane estimated from data reported by Lawrence and McCarty [59] are as follows:

q_{CH_4} = 3.6 mg CH_4-COD/mg VSS-day (acetic acid)

q_{CH_4} = 4.1 mg CH_4-COD/mg VSS-day (propionic acid)

q_{CH_4} = 4.3 mg CH_4-COD/mg VSS-day (butyric acid)

The highest observed methane conversion rate in this study (9.03 mg CH_4-COD VSS-day) is 110 to 150 percent greater than any rates reported or estimated from published data for acetate, propionate and butyrate. The maximum reported rates for several other anaerobic systems are summarized in Table XVI.

The maximum rate of methane production ($\dot{q}_{CH_4}$) can be estimated from the maximum substrate COD removal rate ($\dot{q}$) corrected for cell synthesis, if it is assumed that assimilated COD is converted only to methane or cell COD. If the k_d term is neglected at the maximum substrate removal rate, the correction for cell synthesis is equal to the product of the yield constant (mg VSS/mg COD) and a conversion factor for the COD of cells (1.42 g COD/g VSS [57]). The maximum rate of methane production ($\dot{q}_{CH_4}$) in the hydrogen enrichment systems was estimated to be 24.8 - (1.42)(0.043) or 24.7 mg CH_4-COD/mg VSS-day (based on the maximum rate of hydrogen removal reported in Table X).

TABLE XVI

MAXIMUM METHANE COD PRODUCTION RATES

Substrate	Estimated Rates		Reference
	mg Substrate Utilized per mg Volatile Solids in Reactor - day	$\hat{q}_{CH_4}$, mg CH_4-COD Produced per mg Volatile Solids in Reactor - day	
Hydrogen		$24.7^{a,b,c}$	This Study
Mixed		$0.81^{b,c}$	Andrews et al. [40]
Mixed		$0.92^{b,c}$	Agardy et al. [38]
Acetic Acid Propionic Acid Butyric Acid Total	0.05 0.01 0.08	0.053 0.015 0.145 0.213	Schulze and Raju [66]
Formic Acid Acetic Acid Propionic Acid Butyric Acid Total	0.022 0.017 0.006 0.008	0.008 0.018 0.009 0.014 0.049	Keefer and Urtes [31,32]
Acetic Acid	3.74	$3.80^{b,c}$	Speece and McCarty [58]
Acetic Acid Propionic Acid Butyric Acid	8.7 7.7 8.3	3.6^{b} 4.1^{b} 4.3^{b}	Lawrence and McCarty [59]

[a] Hydrogen COD basis. [b] Based on volatile suspended solids instead of volatile solids.

[c] Corrected for cell synthesis.

Summary

The results of this study show that hydrogen was the growth limiting nutrient in the enrichment fermentations and that the growth and kinetic characteristics of the hydrogen assimilating methane bacteria can be described by the kinetic constants of the Michaelis-Menten substrate removal and the cell continuity equations (Equations 6 and 7 respectively) as reported in Table X.

COD material balances show that about 94 percent of the total COD uptake in the enrichment fermentations was hydrogen COD and six percent was soluble COD; methane accounted for about 93 percent of the product COD, and cell synthesis for the remaining seven percent of the product COD. The percent conversion of assimilated hydrogen and soluble COD to methane showed no significant correlation with cellular residence time.

The generation times of the hydrogen enrichment organisms (13.2 and 15.6 hours) are about one-third to one-fourth the minimum generation times reported by Lawrence and McCarty [59]. The minimum generation times for methanogenic bacteria grown on any kind of substrate in enrichment culture were observed in this investigation. The yield coefficients for the hydrogen assimilating methane bacteria determined in this study were essentially the same as the yield coefficients reported by Lawrence and McCarty [59], that is, from 0.040 to 0.047 mg VSS/mg COD removed.

The hydrogen assimilating methane bacteria are capable of a reported maximum methane production rate of 9.03 mg CH_4-COD/mg VSS-day, which was at least two times greater than the maximum unit rates of methane production estimated from the data of Lawrence and McCarty [59].

Hydrogen Supplemented Fermentations

Two additional continuous culture reactor systems (Systems 3 and 4) were operated to determine the effect of hydrogen on the methane fermentation of volatile acids. Hydrogen was added to each of the reactors for periods of about two hydraulic residence times, which was not sufficient to obtain steady-state in the reactors. Consequently kinetic constants were not calculated.

Results

Rates (mg/day), rate coefficients (mg COD/mg VSS-day), and cell concentrations for hydrogen COD and soluble COD assimilation and methane COD production are summarized in Tables XVII and SVIII for Systems 3 and 4 respectively. Data on experimental flow rates, hydraulic and cell residence times, liquid substrate and volatile acid concentrations, and gas analysis and production rates are summarized in Table XXX (System 3 and Table XXXI (System 4).

The variation with time of effluent hydrogen concentrations, cellular substrate COD loading velocities, cell concentration, and soluble COD removal and methane COD production rate for Systems 3 and 4 is shown in Figure 18, and changes in acetic and propionic acid concentrations, ratio of acetic acid to cell concentrations, effluent substrate concentrations (S_1), and the efficiency of soluble COD removal with time are shown in Figure 19.

TABLE XVII

SYSTEM 3 GROWTH AND KINETIC DATA

Balance Period	H_2-COD Removed mg/day	Soluble COD Removed mg/day	CH_4-COD Produced mg/day	X_1 [a] mg/ℓ	q_{H_2} [b]	q_L [c]	q_T [d]	q_{CH_4} [e]	$(L_c)_L$ [f]	E_L [g] %
1	-	1,380	60	320	-	0.849	0.847	0.036	1.039	81.6
2	-	920	670	380	-	0.485	0.485	0.352	0.679	71.4
3	-	1,340	790	400	-	0.667	0.667	0.393	0.862	77.4
4	-	1,030	850	430	-	0.477	0.477	0.397	0.678	70.4
5	-	1,170	770	510	-	0.453	0.453	0.299	0.589	76.8
6	-	1,250	1,240	640	-	0.393	0.393	0.389	0.478	82.3
7	350	1,180	970	700	0.101	0.351	0.452	0.280	0.434	78.6
8	340	1,620	1.000	690	0.099	0.471	0.570	0.293	0.586	80.2
9	350	1,580	930	680	0.104	0.467	0.571	0.272	0.615	75.8
10	360	1,140	960	790	0.091	0.291	0.382	0.249	0.437	66.6
11	380	1,250	1,000	920	0.082	0.272	0.354	0.217	0.412	66.2
12	370	1,200	1,000	960	0.077	0.250	0.327	0.219	0.390	64.2
13	-	1,000	660	970	-	0.205	0.205	0.134	0.327	62.7
14	-	1,030	750	1,040	-	0.197	0.197	0.145	0.319	61.9
15	-	1,030	940	1,120	-	0.183	0.183	0.167	0.294	62.3
16	-	1,180	1,160	1,160	-	0.203	0.203	0.200	0.295	68.5
17	-	1,210	1,060	1,150	-	0.211	0.211	0.185	0.300	70.2
18	-	1,220	980	1,150	-	0.212	0.212	0.170	0.293	72.3
19	-	1,230	940	1,150	-	0.215	0.215	0.165	0.294	73.0
20	-	1,070	1,040	1,140	-	0.187	0.187	0.183	0.288	65.0
21	-	1,370	870	1,180	-	0.232	0.232	0.147	0.281	82.3
22	-	1,180	1,030	1,160	-	0.205	0.205	0.179	0.292	70.0
23	-	1,320	1,260	1,140	-	0.230	0.230	0.220	0.287	80.2
24	-	1,400	1,290	1,170	-	0.239	0.239	0.220	0.297	80.7
25	-	1,440	1,310	1.170	-	0.247	0.247	0.224	0.298	82.8
26	-	1,451	1,050	1,170	-	0.249	0.249	0.180	0.293	84.8
27	-	1,214	880	1,100	-	0.222	0.222	0.160	0.275	80.8

[a] X_1 = reactor sample VSS.

[b] q_{H_2} = mg H_2-COD removed/mg VSS-day.

[c] q_L = mg soluble COD removed/mg VSS-day.

[d] q_T = mg total (H_2 plus soluble) COD removed/mg VSS-day.

[e] q_{CH_4} = mg CH_4-COD produced/mg VSS-day.

[f] $(L_c)_L$ = mg soluble COD added/mg VSS-day.

[g] E_L = efficiency of soluble COD removal.

TABLE XVIII

SYSTEM 4 GROWTH AND KINETIC DATA

Balance Period	H_2-COD Removed mg/day	Soluble COD Removed mg/day	CH_4-COD Produced mg/day	X_1 [a] mg/ℓ	q_{H_2} [b]	q_L [c]	q_T [d]	q_{CH_4} [e]	$(L_c)_L$ [f]	E_L [g] %
1	-	1,290	100	300	-	0.862	0.862	0.064	1.043	82.6
2	-	1,040	710	350	-	0.587	0.587	0.399	0.767	76.7
3	-	1,350	740	470	-	0.577	0.577	0.316	0.765	75.2
4	-	1,140	980	500	-	0.456	0.456	0.390	0.598	76.3
5	-	1,110	920	570	-	0.391	0.391	0.324	0.544	71.9
6	-	1,140	1,120	750	-	0.306	0.306	0.300	0.418	73.3
7	-	1,270	1,140	800	-	0.341	0.341	0.284	0.416	82.0
8	-	1,650	1,190	830	-	0.432	0.432	0.288	0.498	86.7
9	-	1,890	1,530	920	-	0.435	0.435	0.352	0.496	87.8
10	-	1,660	1,330	1,030	-	0.358	0.358	0.286	0.397	89.7
11	-	1,640	1,170	1,070	-	0.319	0.319	0.226	0.382	83.6
12	-	1,600	1,200	1,080	-	0.297	0.297	0.224	0.355	83.7
13	-	1,540	1,120	1,090	-	0.281	0.281	0.206	0.346	81.0
14	-	1,640	1,100	1,170	-	0.281	0.281	0.188	0.352	79.8
15	-	1,580	1,110	1,290	-	0.246	0.246	0.172	0.316	78.0
16	-	1,530	1,090	1,390	-	0.221	0.221	0.157	0.294	75.0
17	-	1,560	1,090	1,530	-	0.204	0.204	0.143	0.278	73.5
18	-	1,490	1,080	1,580	-	0.189	0.189	0.137	0.260	72.6
19	420	1,440	1,100	1,580	0.053	0.182	0.235	0.139	0.258	70.3
20	400	1,330	970	1,520	0.053	0.175	0.228	0.127	0.263	66.7
21	380	1,160	1,040	1,480	0.052	0.157	0.209	0.141	0.257	61.2
22	390	1,230	1,200	1,400	0.056	0.176	0.232	0.172	0.289	60.8
23	390	1,160	1,050	1,280	0.061	0.183	0.244	0.166	0.308	59.5
24	390	1,170	1,080	1,280	0.060	0.183	0.243	0.168	0.313	58.3
25	-	1,200	830	1,290	-	0.187	0.187	0.139	0.317	58.8
26	-	1,140	690	1,280	-	0.178	0.178	0.109	0.304	58.5
27	-	980	680	1,260	-	0.156	0.156	0.108	0.285	54.7

[a] X_1 = reactor sample VSS.

[b] q_{H_2} = mg H_2-COD removed/mg VSS-day.

[c] q_L = mg soluble COD removed/mg VSS-day.

[d] q_T = mg total (H_2 plus soluble) COD removed/mg VSS-day.

[e] q_{CH_4} = mg CH_4-COD produced/mg VSS-day.

[f] $(L_c)_L$ = mg soluble COD added/mg VSS-day.

[g] E_L = efficiency of soluble COD removal.

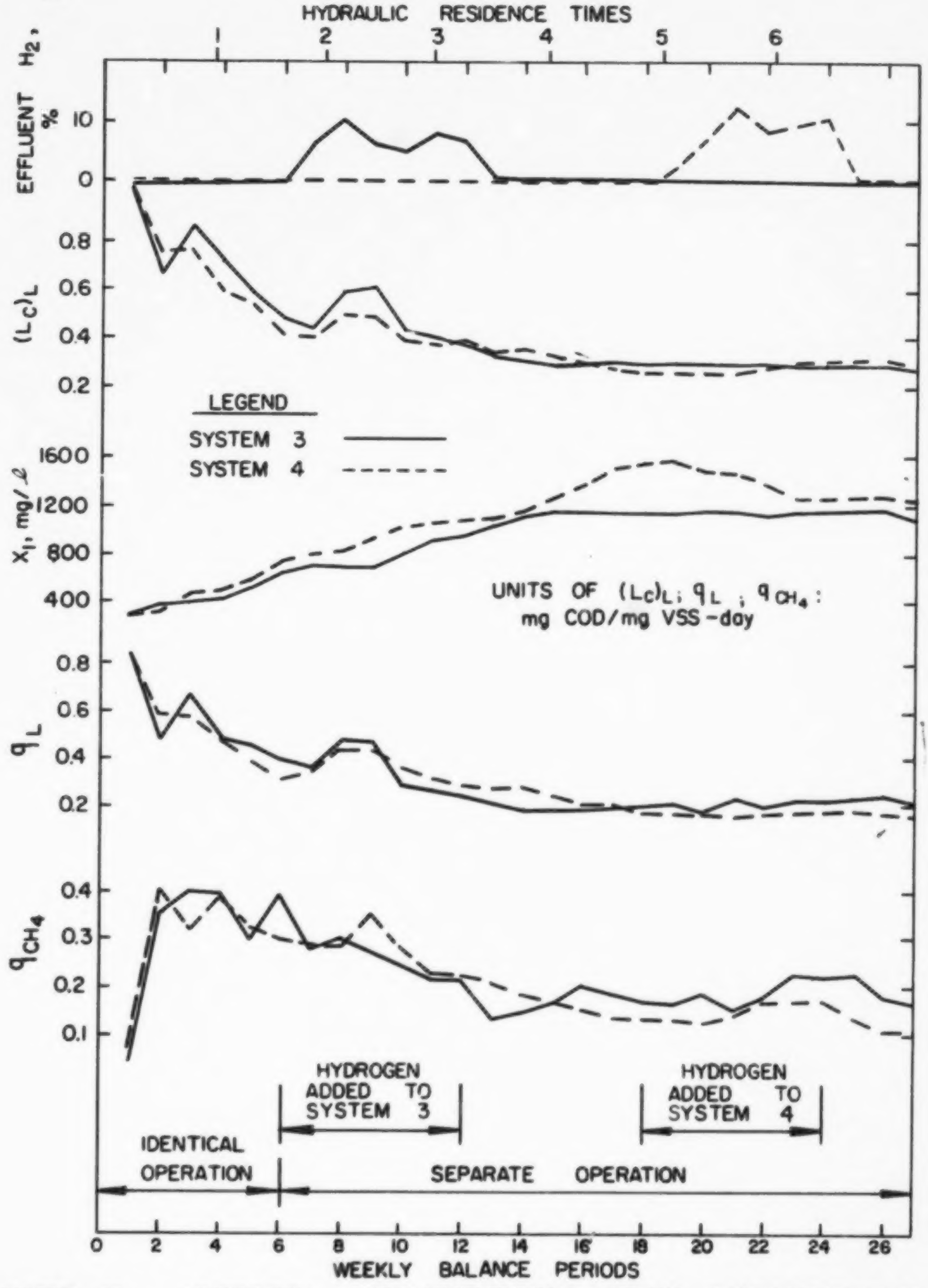


FIGURE 18. HYDROGEN SUPPLEMENTATION STUDY CHARACTERISTICS

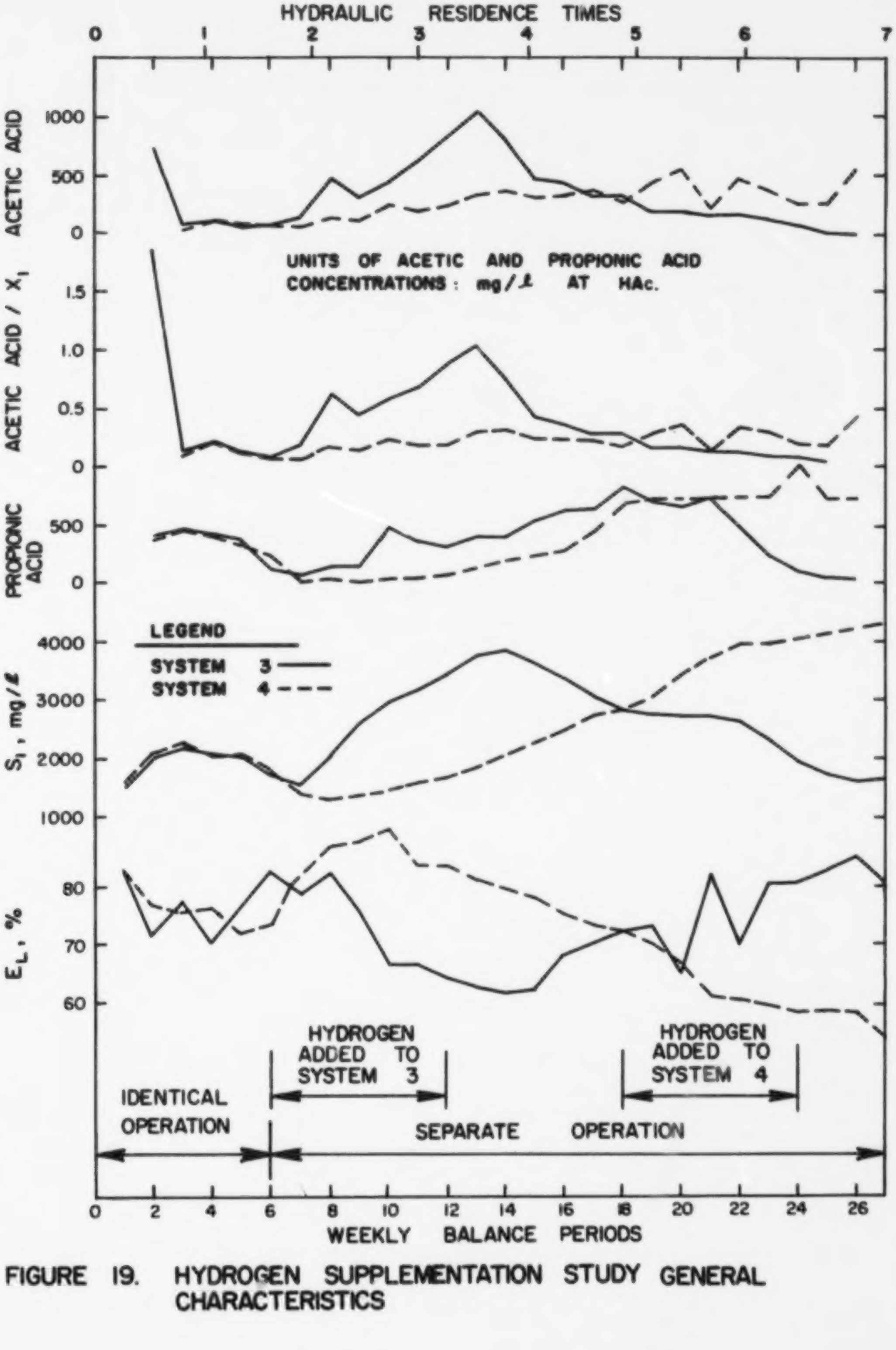


FIGURE 19. HYDROGEN SUPPLEMENTATION STUDY GENERAL CHARACTERISTICS

Culture Homogeneity.--The liquid contents of the reactors of Systems 3 and 4 were mixed with each other during BP (Balance Periods) 1 to 6 to establish homogeneity of the anaerobic cultures prior to hydrogen addition. The acetic and propionic acid concentrations of both reactors decreased during BP 1 to 6, and were similar in each reactor at the end of BP 6.

The rates of soluble COD removal and methane COD production (mg COD/day) were about 10 percent greater in Reactor 3 than in Reactor 4 at BP 6. However, the VSS concentration in Reactor 4, which was the same as the concentration as found in Reactor 3 at BP 1, increased 17 percent over that found in Reactor 3 at BP 6, which indicated that the mass rate of transfer of VSS between Reactors 3 and 4 was not uniform. The soluble COD removal rates (q_L) and methane COD production rates (q_{CH_4}) were over 20 percent greater in Reactor 3 than in Reactor 4 at BP 6, apparently due to the lower VSS concentration observed in Reactor 3 at BP 6. It appears that the mixing procedure used with Reactors 3 and 4 between BP 1 and 6 did not develop two anaerobic cultures of similar cell (VSS) concentrations, although essentially the same levels of acetic and propionic acids were observed at BP 6 in either reactor.

Hydrogen Supplementation.--Hydrogen was added to Reactor 3 between BP 7 and 12, and to Reactor 4 between BP 19 and 24. Unassimilated hydrogen was detected in the effluent gases of Reactor 3 from BP 7 to 13, and in Reactor 4 from BP 19 to 24, as shown in Figure 18. The substrate COD concentration in the liquid feed to both reactors was increased about 25 percent to approximately 10,000 mg/l at the start of BP 8. Because VSS concentrations in both Reactors 3 and 4 were increasing about 10 percent per BP at BP 8, the effect of the increased substrate COD concentration on the load coefficients $(L_c)_L$ of Reactors 3 and 4 could not be detected after two balance periods.

The acetic acid concentration of Reactor 3 increased nearly 1,600 percent (from 60 to 1,030 mg/l) between BP 6 to BP 13, during which time hydrogen reached a concentration of 10 percent in the reactor gases. Thereafter the acetic acid concentration of Reactor 3 decreased to about 50 mg/l at BP 26. However, the acetic acid concentration in Reactor 4 increased less than 85 percent (from 300 to 550 mg/l) during hydrogen addition, and showed no consistent decrease thereafter. Propionic acid concentrations increased in both reactors in a trend which did not change with the start or termination of hydrogen addition.

The effluent soluble COD concentration in each reactor reflected the concentrations of acetic and propionic acid of the reactor, as shown in Figure 19. The effluent soluble COD concentration increased (and the efficiency of soluble COD removal decreased) almost continuously in Reactor 4 after BP 10, and in Reactor 3 from BP 6 to 13 during hydrogen addition). The effluent soluble COD concentration in Reactor 3 decreased after hydrogen disappeared from the reactor gases.

Changes in the soluble or total (hydrogen plus soluble) COD removal rates were not correlated with the presence of hydrogen in the reactor; this may have been caused at least in part by the increasing VSS concentrations. However, the methane production rate coefficient (q_{CH_4}, mg CH_4-COD/mg VSS-day) and the rate (mg CH_4-COD/day) decreased in both reactors as hydrogen concentrations decreased in the reactors. The decreases in methane production rates (about 400 mg CH_4-COD/day, or 35 percent) were essentially equal to the rates of hydrogen assimilation during hydrogen addition.

Discussion

The performance of Reactors 3 and 4 during hydrogen addition was characterized by a similar and consistent decrease in the efficiency at which both fermentations converted organic substrate to methane. However, there was significantly greater increase of acetic acid (~1,600 percent or 1,000 mg/ℓ) in Reactor 3 than in Reactor 4 (~ 85 percent or 250 mg/ℓ) during hydrogen addition; and the different fermentation conditions in Reactors 3 and 4 at the start of hydrogen addition may explain why acetic acid increased in Reactor 3 but not in Reactor 4. The acetic acid concentration was 300 mg/l in Reactor 4 but only 60 mg/l in Reactor 3 at the beginning of hydrogen addition. The propionic acid concentration was 690 mg/l in Reactor 4 but about 145 mg/l in Reactor 3, and the effluent soluble COD concentration was 2,800 mg/l in Reactor 4 and 1,700 mg/l in Reactor 3. Moreover, the rate coefficients based on soluble COD loading, soluble COD removal, and methane COD production were over twice as large in Reactor 3 as in Reactor 4 at the start of hydrogen addition. The acetic and propionic acid concentrations observed in Reactor 3 at the start of hydrogen addition (BP6) indicate a more active methane fermentation than do the corresponding characteristics of Reactor 4 at the same stage with respect to hydrogen addition. Thus, the changes in Reactor 3 during hydrogen addition would be expected to reflect more accurately the effect of hydrogen on the methane fermentation of volatile acid.

Unfortunately, the impact of the increased soluble COD concentration of the feed (BP 8) in either reactor cannot be separated from that due to hydrogen addition. The 25 percent increase in feed concentration at BP 8 increased the loading coefficients for only two balance periods before cell concentrations had increased sufficiently to reduce the loading coefficients below the levels at BP 8. The increased soluble COD concentrations at BP 8 in both reactors may have caused the accumulation of volatile acids which occurred in both reactors either alone or in combination with the effects of hydrogen. However, the hypothesis that hydrogen, rather than increased feed concentration, is the principal cause of accumulation of volatile acids appears to be valid because:

1. Acetic acid decreased rapidly in Reactor 3 after hydrogen addition was terminated. Such depletion would be coincidental if hydrogen did not cause the accumulation.
2. The rate of soluble COD removal (mg/day) appeared to decrease in an amount approximately equal to the hydrogen COD removal.

Additional work is required to show whether the accumulation of hydrogen as an intermediate byproduct of glycolytic or other fermentations in a methane fermentation is the cause of volatile acid accumulation. The results of this study suggest that hydrogen supplementation may have caused the accumulation, and that increasing concentrations of hydrogen in a methane fermentation (which can be measured accurately in concentrations of less than one percent with gas chromatography) may be an indicator of oncoming digester failure.

V. CONCLUSIONS

The specific objectives of this investigation were as follows:

1. To determine the microbiological kinetic constants of the methane bacteria which ferment hydrogen.
2. To evaluate experimentally the effect of hydrogen on the methane fermentation of volatile acids.

The conclusions developed from this investigation are as follows:

1. The results of the growth and kinetic study indicate that a continuous flow enrichment culture predominant in the hydrogen assimilating methane bacteria can well be characterized by the Michaelis-Menten model and the cell continuity equation as follows:

$$\frac{1}{\theta_c} = Yq - k_d$$

$$q = \frac{\hat{q}\,(S_1)_G}{K_s + (S_1)_G}$$

The following growth and kinetic constants were observed.

a. Hydrogen COD basis

$$Y = 0.043 \frac{\text{mg VSS}}{\text{mg } H_2 - \text{COD removed}}$$

$$k_d = -0.009 \text{ day}^{-1}$$

$$\hat{q}_{H_2} = 24.8 \frac{\text{mg } H_2 \text{ COD removed}}{\text{mg VSS - day}}$$

$$K_s = 569 \text{ mm Hg}$$

b. Total (hydrogen plus soluble) COD basis

$$Y = 0.040 \frac{\text{mg VSS}}{\text{mg Total COD removed}}$$

$$k_d = -0.011 \text{ day}^{-1}$$

$$\hat{q}_T = 31.7 \frac{\text{mg Total COD removed}}{\text{mg VSS - day}}$$

$$K_s = 724 \text{ mm Hg}$$

2. The maximum rate of substrate COD removal ($\hat{q}_{H_2}$ = 24.8 mg H_2-COD/mg VSS-day) of the hydrogen assimilating methane bacteria in the enrichment systems was twice the calculated maximum rate of hydrogen COD removal for a pure culture of the methane bacterium M. omelianskii as reported by Barker [29].

3. Maximum growth rates and minimum generation times of the hydrogen assimilating methane bacteria were calculated from the growth and kinetic constants as follows:

   a. Hydrogen COD basis:

   $\hat{\mu}$ = 1.06 days

   g = 15.6 hours

   b. Total COD basis:

   $\hat{\mu}$ = 1.26 days^{-1}

   g = 13.2 hours

   The calculated generation times of the methane bacteria which ferment hydrogen appears to be about one-half that reported previously for methane bacteria grown in enrichment culture (methanol substrate; g = 1.4 days).

4. The maximum observed rate of methane COD production was as follows:

$$q_{CH_4} = 9.03 \frac{\text{mg } CH_4\text{-COD}}{\text{mg VSS-day}}$$

This rate is from 110 to 150 percent greater than that reported for species of the methane bacteria grown in enrichment cultures on acetic, propionic, and butyric acid substrates.

The maximum calculated rate of methane COD production, $\hat{q}_{CH_4}$, obtained by correcting the maximum hydrogen COD removal rate for cellular synthesis, was as follows:

$$q_{CH_4} = 24.7 \frac{\text{mg } CH_4\text{-COD}}{\text{mg VSS-day}}$$

5. Although the methane bacteria which ferment hydrogen are capable of high substrate conversion rates, the rate of hydrogen assimilation by these bacteria in a normal sludge digester is expected to be less than a few percent ($\sim$3) of the maximum hydrogen assimilation rates measured in this study.

6. The addition of hydrogen to an active methane fermentation of volatile acids produced the following conditions in the fermentation:
    a. An increase in the acetic acid concentration.
    b. A decrease in the efficiency of removal of organic substrate.
    c. A decrease in methane production from the organic substrate.

Consequently it appears that the addition of hydrogen to a methane fermentation such as in a sludge digestion would not produce beneficial results.

GLOSSARY

The symbols of this research conform with the "Unified Fundamental Symbols for Continuous Culture of Microorganisms" [67] insofar as possible. Slight modifications have been made where required to suit the needs of this research. The symbols are presented in order of appearance.

q	Substrate removal rate coefficient, mass of substrate removed per mass of organisms--time.
$\hat{q}$	Maximum substrate removal rate coefficient, a constant.
K_S	Saturation constant, or the substrate concentration at one-half the maximum substrate removal metabolic coefficient or growth rate, mass per volume.
S_1	Concentration of limiting nutrient, mass per volume.
θ_c	Cell residence time.
Y	Yield coefficient, mass of cell produced per mass of substrate removed.
k_d	Specific organism decay rate, time.$^{-1}$
μ	Specific growth rate, time.$^{-1}$
$\hat{\mu}$	Maximum specific growth rate, time.$^{-1}$
g	Generation or doubling time of the organism.
θ_W	Critical or washout residence time.
θ	Hydraulic residence time.
X_1	Reactor organism concentration, mass per volume.
X_2	Separator effluent organism concentration, mass per volume.

W	Weight of the acid reservoir for each substrate gas reservoir.
ρ	Density of aqueous 3.13 percent ($^w/w$) H_2SO_4 solution, 1.021 g/cu cm.
$\bar{P}$	Average sum of the total (atmospheric plus manometric differential) pressures in the substrate gas reservoir at the beginning and end of a balance period, mm Hg.
P_v	Vapor pressure of water over the container liquid at temperature T, mm Hg.
$\bar{T}$	Average of temperatures at the beginning and end of a balance period, °K.
t	Duration of balance period, days.
P	Atmospheric pressure, mm Hg.
T	Temperature, °K.
V_L	Volume of liquid displaced by fermentation gases, liters.
h	Height of column of liquid suspended above the liquid outlet level, mm.
$(V_D)_{H_2}$	Volume of hydrogen (at 0°C and 760 mm Hg) diffusing per day per atmosphere of hydrogen.
$(V_D)_{CH_4}$	Volume of methane (at 0°C and 760 mm Hg) diffusing per day per atmosphere of methane.
q_{H_2}	Hydrogen removal rate coefficient, mg H_2-COD removed per mg VSS-day.
G_0	Influent hydrogen flow rate, mg H_2-COD per day.
G_1	Effluent hydrogen flow rate (from both effluent gas and gas sample streams), mg H_2-COD per day.
V	Reactor volume.
q_L	Soluble COD removal rate coefficient, mg soluble COD removed per mg VSS-day
F_0	Liquid influent flow rate, liters per day.
F_1	Reactor effluent flow rate, liters per day.
F_s	Reactor sample flow rate, liters per day.

$(S_0)_L$	Influent soluble COD concentration, mg COD per liter.
$(S_1)_L$	Reactor and effluent soluble COD concentration, mg COD per liter.
q_T	Total COD removal rate coefficient, mg total (H_2 plus soluble) COD removed per mg VSS-day.
$(L_C)_{H_2}$	Hydrogen COD loading coefficient, mg H_2-COD per mg VSS-day
E_{H_2}	Efficiency of hydrogen COD removal, percent.
$(L_C)_L$	Soluble COD loading coefficient, mg soluble COD per mg VSS-day.
E_L	Efficiency of soluble COD removal, percent.
$(L_C)_T$	Total COD loading coefficient, mg total COD per mg VSS-day.
E_T	Efficiency of total COD removal, percent.
$(S_1)_G$	Reactor hydrogen partial pressure, mm Hg.
q_{CH_4}	Methane production rate coefficient, mg CH_4-COD produced per mg VSS-day.
V_G	Volume of fermentor gas at standard temperature and pressure, liters.
P_e	Permeability of gas, liters/day per sq. sm. per atm/cm thickness.
v_D	Volume of gas (at 0°C and 760 mm Hg) diffusing per day per sq. cm. solid surface.
A	Average cross section for diffusion, sq. cm.
dp/dz	Pressure gradient, atm/cm thickness.
r_1	Inner radius, cm.
r_2	Outer radius, cm.
1	Length of tubing, cm.
$(P_e)_{H_2}$	Permeability of hydrogen, liters/day per sq. cm. per atm/cm thickness.
$(P_e)_{CH_4}$	Permeability of methane, liters/day per sq. cm. per atm/cm thickness.

APPENDICES

APPENDIX A

CHARACTERISTICS OF THE SUBSTRATE

Bacto-Beef Extract (Difco Laboratories)

Item	Composition %
Total solids	80.00
Ash in total solids	19.80
Chlorides as NaCl	4.05
Total nitrogen	8.67
Ammonia nitrogen	0.16
Peptone nitrogen	2.30
Proteose nitrogen	1.20
Total meat base nitrogen	5.05
Nitrates	Nil
Tyrosine	Nil
Cystine	0.13
Creatin	1.50
Creatinin	1.50
Iron	Nil

Bacto-Yeast Extract (Difco Laboratories)

Item	Composition%
Ash	10.0
Total Nitrogen	9.5
Amino Nitrogen	7.0
Arginine	1.0
Aspartic Acid	5.0
Glutamic Acid	6.5
Glycine	2.5
Histidine	1.0
Isoleucine	3.0
Leucine	3.5
Lysine	4.0
Methionine	1.0
Phenylalanine	2.0
Threonine	3.5
Tyrosine	0.5
Valine	3.5

Plus water soluble factors and vitamins.

Bacto-Tryptone (Difco Laboratories)

Item	Amount
Ash	7.28 %
Total nitrogen	13.14 %
Chloride	0.29 %
Sulfur (Total)	0.57 %
Lead	6.00 μg/g
Arsenic	0.07 μg/g
Manganese	13.20 μg/g
Zinc	30.00 μg/g
Phosphorus	0.75 %
Iron	0.0071%
Copper	16.00 μg/g
SiO_2	0.090 %
Potassium	0.30 %
Sodium	2.69 %
Magnesium	0.045 %
Calcium	0.096 %
Arginine	3.30 %
Aspartic acid	6.40 %
Glutamic acid	18.90 %
Glycine	2.40 %
Histidine	2.00 %
Isoleucine	4.80 %
Leucine	8.50 %
Lysine	6.80 %
Methionine	2.40 %
Phenylalanine	4.10 %
Threonine	3.10 %
Tryptophane	1.45 %
Tyrosine	7.10 %
Valine	6.30 %

Plus water soluble factors and vitamins.

APPENDIX B

DETERMINATION OF FERMENTATION GASES
BY GAS CHROMATOGRAPHY

Equipment

The equipment and the program of operation for the chromatograph are summarized in Table XX. Only proprietary equipment is used.

Method

Principles of Operation and Choice of Carrier Gas

The thermal conductivity detector used with the selected equipment is dependent upon the rate of heat removal by the gas mixture from a hot body, a rate which is a function of the composition of the gas surrounding the heated body (McNair and Bonelli [68]. As the carrier gas of the chromatograph is passed through the reference filaments and the sample gas through identical detecting filaments, a small current is generated by the differential cooling between the two filaments and recorded as a peak on a potentiometric recorder.

The thermal conductivity detector requires careful selection of the carrier gas to be used in the analysis. The ability of a gas to transfer heat is proportional to its thermal conductivity; optimization of detector response requires selection of a carrier gas with the maximal thermal conductivity differential between it and the sample gas. The relative thermal conductivity factors of several gases are given in Table XXI (Littlewood [55], McNair [68]).

The basis for selection of helium as carrier gas for the analysis of carbon dioxide, oxygen, nitrogen and methane is apparent in Table XXI. Nitrogen is selected as the carrier gas for hydrogen analysis rather than carbon dioxide because nitrogen is inert. Additionally, carbon dioxide is irreversibly retained on the external molecular sieve column at room temperature, although it can be removed from the external column by an overnight flushing with nitrogen at 110°C [55]. Water-pumped nitrogen is a satisfactory carrier for the analysis of hydrogen.

TABLE XX

EQUIPMENT AND PROGRAM OF OPERATION

Chromatograph	Aerograph A-90-P3 Chromatograph with thermal conductivity detector (Varian Aerograph, Walnut Creek, California)
Sample Introduction and Volume	Linear gas sampling valve, 6-way; 1 ml sample size (Varian Aerograph)
Recorder	Leeds and Northrup Speedomax W, 1/2 inch/minute chart speed, with #9224 Disc Integrator (Disc Instruments, Inc., Santa Ana, California)
Columns	Internal: 21" x 1/4" O.D. pyrex, of silica gel with no solid support (Varian Aerograph)
	External: 15' x 1/4" O.D. copper, of 100 per cent Molecular Sieve 13X (Varian Aerograph)
Column Temperatures	Internal column: 115°C
	External column: 22°C (room temperature)
Detector Temperature	90°C
Injector Temperature	Potentiometer in "off" position
Carrier Gases	Nitrogen (hydrogen analysis); helium (carbon dioxide, oxygen, nitrogen, and methane analysis)
Carrier Gas Flow Rate	60 ml/min at 60 psig
Filament Current	Nitrogen carrier: 120 milliamperes
	Helium carrier: 150 milliamperes
Attenuator	1 to 128, depending on gas composition

TABLE XXI

RELATIVE THERMAL CONDUCTIVITY FACTORS

Gas	Relative Thermal Conductivity
Hydrogen	41.6
Helium	34.8
Methane	7.21
Oxygen	5.90
Nitrogen	5.81
Carbon Dioxide	3.39

Column Arrangement

A two column system is employed to achieve full resolution and detection of carbon dioxide, hydrogen, oxygen, nitrogen, and methane. The 21-inch internal silica gel column is used to separate carbon dioxide, but was of insufficient length at the operating temperature utilized (115°C) to resolve hydrogen, oxygen, and nitrogen, and carbon dioxide. The external molecular sieve column is required to complete the resolution of the latter gases, but irreversibly retains carbon dioxide at room temperature. Figure 20 illustrates a column arrangement which performed satisfactorily.

Hydrogen sulfide also can be resolved by this system, but it is not included in the procedure because hydrogen sulfide normally constitutes less than 0.5 percent of digester gas composition.

With the column arrangement of Figure 20, the polarity of the signal from the detector is reversed as gases eluting from the molecular sieve column pass back through the detecting filaments. At this point the reference and detecting filaments become interchanged in function, and a polarity switch is required on the signal cable to the recorder in order to maintain a positive recorder signal after the appearance of the carbon dioxide peak.

Sample Introduction

The six-way gas-sampling valve allows rapid and replicable introduction of the gas sample to the carrier stream, without air contamination. Gas-tight syringes can also be utilized, but were

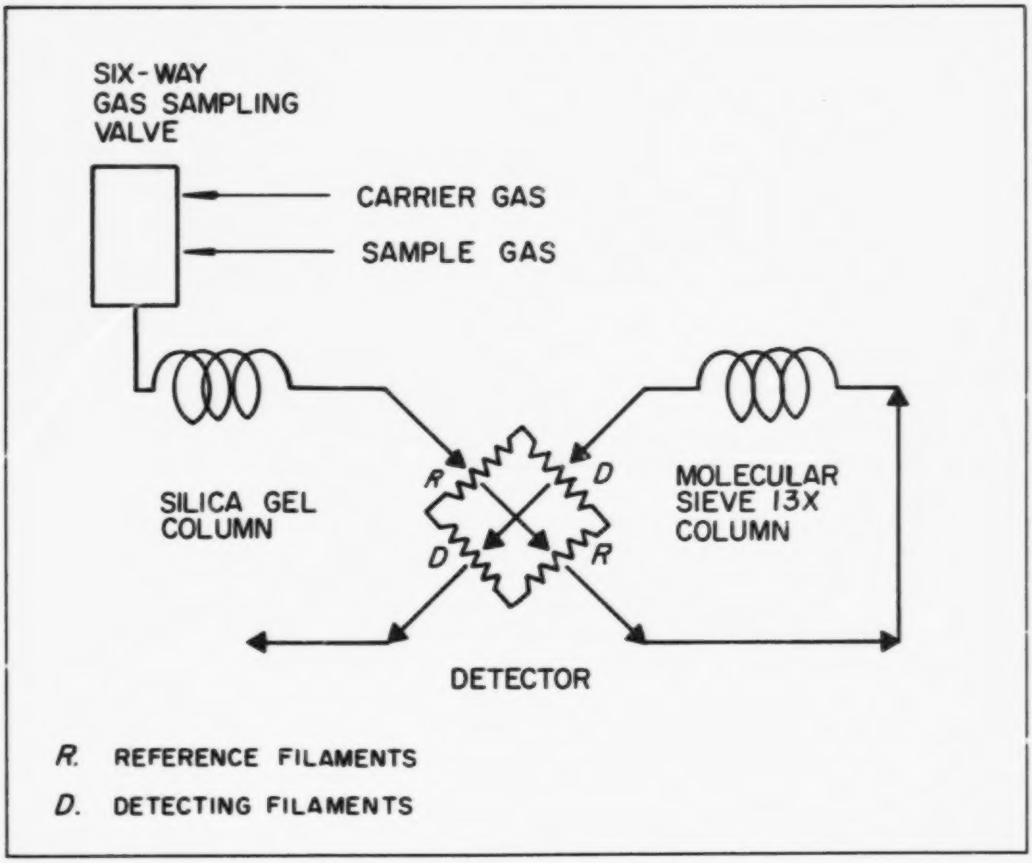

SIX-WAY
GAS SAMPLING
VALVE
CARRIER GAS
SAMPLE GAS
SILICA GEL
COLUMN
R
D
D
R
MOLECULAR
SIEVE 13X
COLUMN
DETECTOR
R. REFERENCE FILAMENTS
D. DETECTING FILAMENTS

FIGURE 20. COLUMN ARRANGEMENT IN CHROMATOGRAPH

found impractical because of difficulties with sample size replication and contamination with air. The gas sample is transferred to the sample valve by displacement with five percent ($^{w}/w$) aqueous sulfuric acid solution flowing into the gas buret containing the sample. After approximately five ml of gas have been flushed through the sampling valve, the valve is closed and a one ml volume of sample automatically injected into the carrier gas stream. Three replicate analyses can be achieved from 10 ml of sample gas with the sampling valve system.

Chromatogram Analysis

Analysis of Results

The components of a gas sample are represented qualitatively by the position of a peak on the time scale, and quantitatively by the area of the peak above the chromatogram base line. Peak areas may be determined by triangular approximation with a scale, by weighing of the chart paper cutout of the peak, or most conveniently by use of an automatic area integrator. The latter method was used in our laboratory.

Two sets of analysis are required for each sample, one with the helium and one with the nitrogen carrier gas. A typical gas analysis chromatogram is shown in Figure 21. The integrator units under each peak are totaled and multiplied by the attenuation of the peak. Each sweep of the integrator pen is arbitrarily measured as 100 integrator units. The utilization of standard calibration factors for each component, expressed as percent composition per thousand integrator units and derived from the analysis of standard gas mixtures, allows calculation of the percent composition of each component in the sample.

Analytical Precision

The statistical accuracy of the analysis was evaluated by analyzing 10 replicate aliquots of samples containing carbon dioxide, hydrogen, oxygen, nitrogen, and methane. The coefficients of variation were determined for each series, and the results are presented in Table XXII. The coefficients of variation for each component gas ranged from a minimum of 0.21 to a maximum of 1.18 percent at the concentrations analyzed which is a level of precision equal to or greater than many conventional chemical analyses.

Gas	Carrier Gas	Attenuation	% Composition
H_2	Nitrogen	64	18.32
CO_2	Helium	2	3.61
O_2	Helium	1	2.53
N_2	Helium	16	57.26
CH_4	Helium	4	18.28

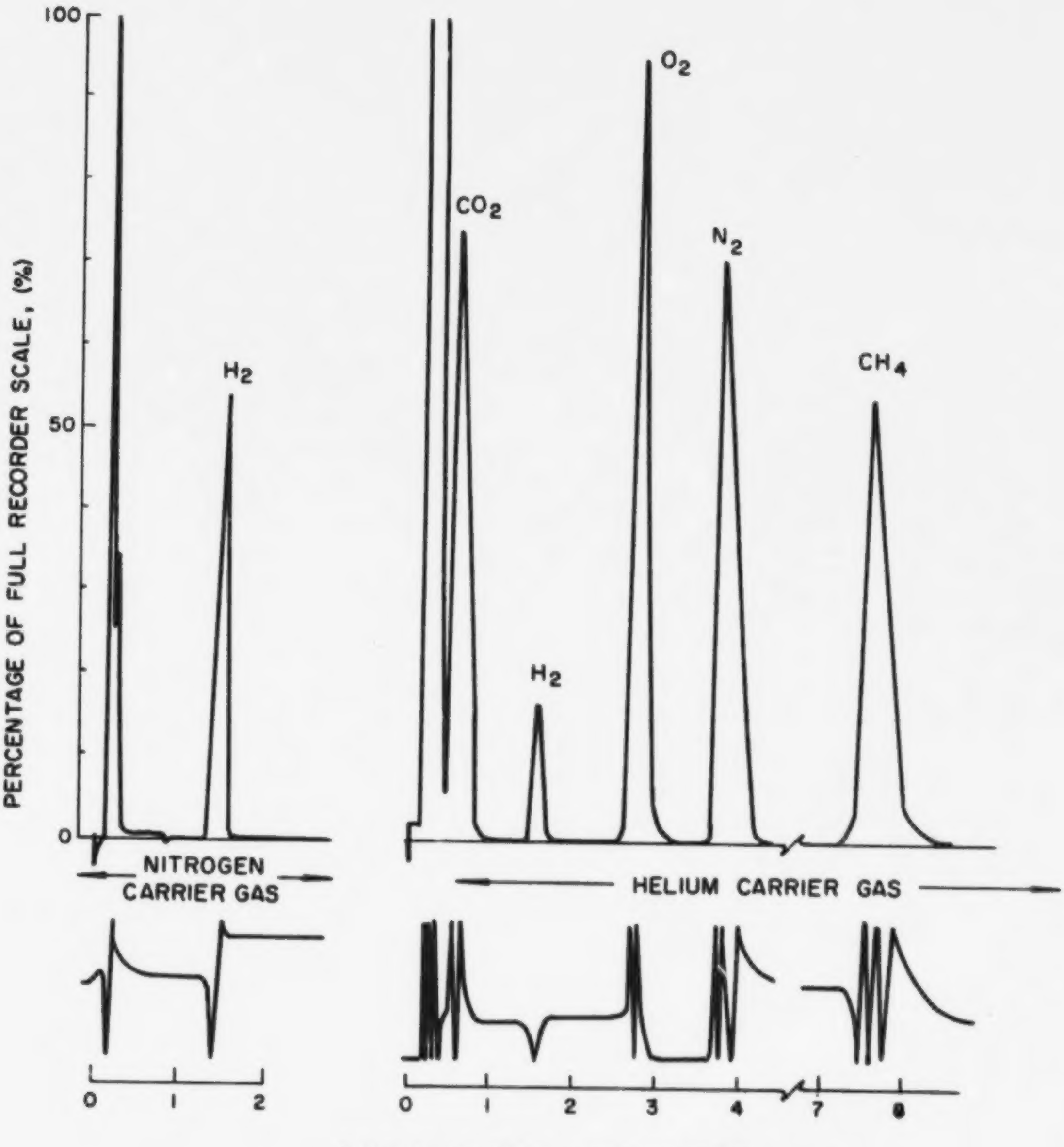


FIGURE 21. TYPICAL GAS ANALYSIS CHROMATOGRAM

TABLE XXII

ANALYTICAL PRECISION WITH STANDARD GAS MIXTURES[a]

Standard Gas Per Cent of Sample Gas (Carrier)	Peak Areas (Integrator Units at an Attenuation of One)				
	H_2 68.94 (N_2)	CO_2 48.61 (He)	O_2 20.95 (He)	N_2 78.08 (He)	CH_4 50.00 (He)
Sample Number					
1	40064	8960	3064	11552	6752
2	40448	8864	3104	11600	6840
3	40448	8960	3104	11552	6792
4	40320	8864	3048	11552	6816
5	40704	8864	3080	11552	6816
6	40192	8832	3072	11664	6832
7	40576	8864	3088	11552	6938
8	40704	8768	3080	11584	6920
9	40576	8704	3080	11616	6952
10	40448	8646	3104	11504	7008
Average	40448	8833	3082	11573	6880
Standard Deviation	209	90	19	245	81
Coefficient of Variation, Per Cent	0.52	1.02	0.62	0.21	1.18
Calibration Factor Per Cent/1000 Integrator Units	1.704	5.503	6.802	6.740	7.267

[a] For operating program, see Table XX.

APPENDIX C

METHOD FOR QUANTITATIVE RECOVERY AND CONTAINMENT OF FERMENTATION GASES

Quantitative recovery and containment of the fermentation gases CO_2, H_2, and CH_4 at near atmospheric pressure has been accomplished by modification of the Mariotte-type liquid-dispensing bottle. Figure 22 illustrates the gas container and its appurtenances, and Table XXIII presents a program of operation for the refilling and containment cycles.

The gas volume enters through the inlet tube (Figure 22) and immediately rises to the top of the bottle (gas storage zone). Here the gas is held at a slightly reduced pressure which equals the atmospheric pressure less the unit force (or pressure) exerted at the gas-liquid interface by the column of liquid suspended above the liquid-outlet level. A bell-shaped inlet and a U-outlet tube are desirable if negative pressures occasionally result in the fermentor system. The pressure necessary to force gases into the container can be adjusted by changing the vertical distance between the inlet and outlet tube. A two mm vertical distance proved satisfactory.

The volume of gas displaced during the containment cycle is proportional to the volume of liquid displaced, corrected by the fractional reduction of atmospheric pressure in the gas storage zone due to (1) vapor pressure of the container fluid and (2) the pressure reduction by the column of suspended fluid. At standard temperature and pressure, the gas volume is calculated as follows:

$$V_G = V_L \left(\frac{273}{760}\right) \frac{P - P_v - 0.0734\rho h}{T} \qquad (30)$$

where:

V_G = Volume of fermentor gas at standard temperature and pressure, liters.

V_L = Volume of liquid displaced by fermentation gases, liters.

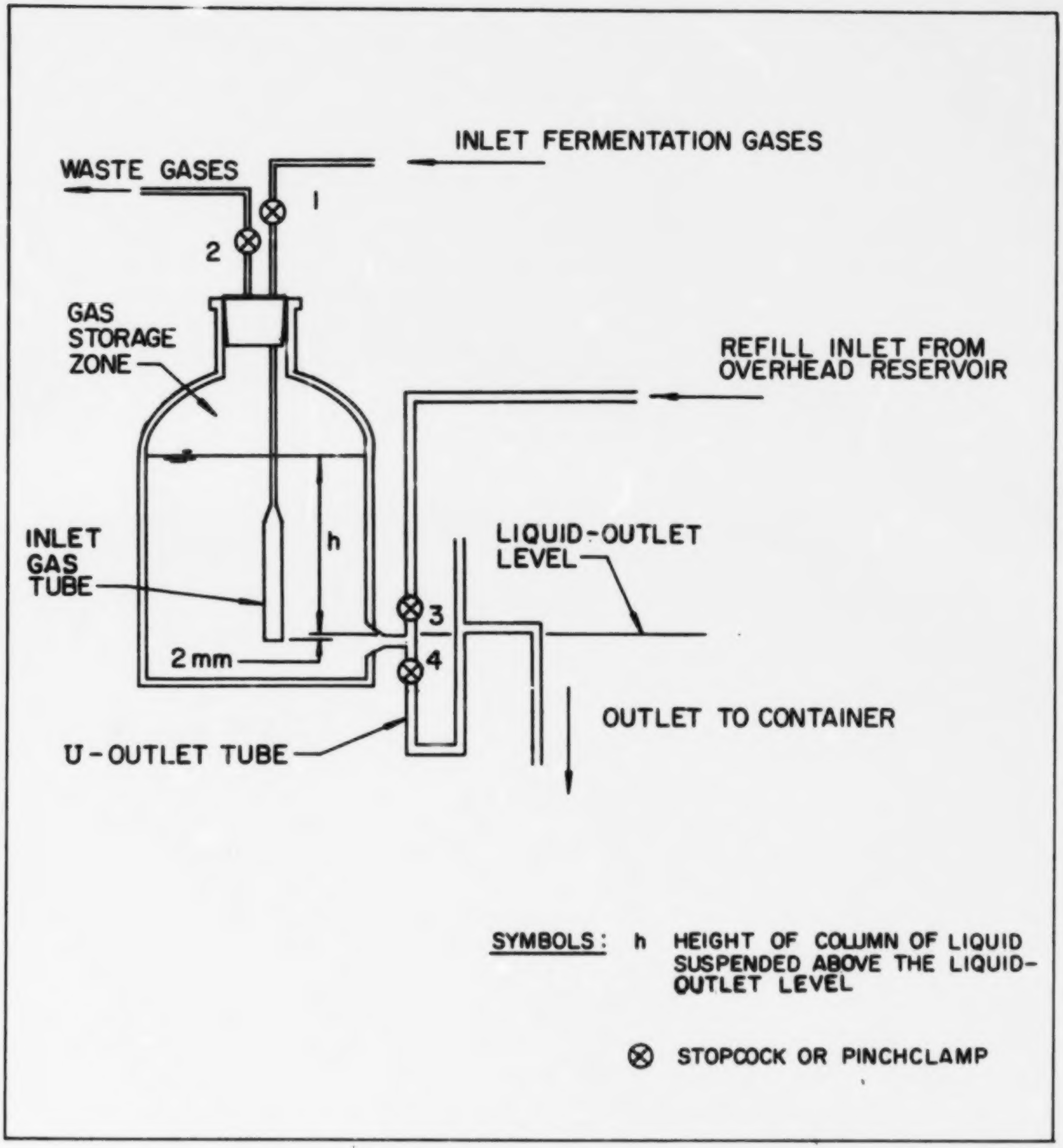

INLET FERMENTATION GASES
WASTE GASES
1
2
GAS STORAGE ZONE
REFILL INLET FROM OVERHEAD RESERVOIR
INLET GAS TUBE
h
LIQUID-OUTLET LEVEL
3
4
2 mm
OUTLET TO CONTAINER
U-OUTLET TUBE
SYMBOLS: h HEIGHT OF COLUMN OF LIQUID SUSPENDED ABOVE THE LIQUID-OUTLET LEVEL
⊗ STOPCOCK OR PINCHCLAMP

FIGURE 22. CONTAINER AND APPURTENANCES

TABLE XXIII

GAS CONTAINER PROGRAM OF OPERATION

Cycle	Operation (see also Figure 22)
Fill or Refill	1. Close valves 1 and 4. 2. Open valve 3. Allow refill liquid from overhead reservoir to slightly compress the gas in the gas storage zone. 3. Open valve 2. Refill reservoir completely. During this refilling period, the slightly compressed gas may be conveniently sampled at the waste gas outlet with minimum chance of air contamination. 4. Close valves 2 and 3. 5. Open valve 4. 6. Empty container for displaced liquid or make a tare reading. 7. Open valve 1 and start new containment cycle.
Containment	1. Valves 1 and 4 are open; valves 2 and 3 are closed. 2. When the liquid level in the container is about one inch above the bottom of the gas inlet tube, or at the end of a containment cycle, the room temperature (T) and pressure (P), the height (h), and the volume of displaced liquid (V_L) are recorded. 3. Proceed to Step 1 of Fill Cycle above.

P = Atmospheric pressure, mm H_g.

P_v = Vapor pressure of water over the container liquid at temperature T, mm H_g.

ρ = Density of container liquid

h = Height of the column of liquid suspended above the liquid outlet level, mm fluid.

T = Temperature, °K.

Aqueous sulfuric acid (3.13 percent $^{w}/w$) of specific gravity 1.021 was found to be a suitable container fluid. The gases carbon dioxide, hydrogen, and methane are relatively insoluble in this solution, and the use of a pH indicator such as methyl red allows rapid visual inspection of fluid levels in the container. Tables of the water vapor pressure: temperature relationships are available for this solution (Perry [69]).

APPENDIX D

DIFFUSION CORRECTION IN HYDROGEN ENRICHMENT STUDY

Theory

Diffusion is the transfer of one substance through another, usually on a molecular scale. Mass transfer results from a concentration difference, the direction of transfer being from a zone of high to one of low concentration.

The diffusion of a gas passing through a solid is characterized by the permeability of the gas in the solid. The permeability is the product of the solubility and the diffusivity of the gas in the solid material, and is defined as follows (after Treybal [56]):

$$P_e = \frac{v_D}{A \cdot dp/dz} \qquad (31)$$

where:

P_e = permeability of gas, liters/day per sq cm per atm/cm thickness.

v_D = volume of gas (at 0°C and 760 mm Hg) diffusing per day per sq cm solid surface.

A = average cross section for diffusion, sq cm.

dp/dz = pressure gradient, atm/cm thickness.

For diffusion through the walls of a tube such a Tygon tubing, the average cross section for radial diffusion (A) is given as below [56]:

$$A = \frac{2\pi l(r_2-r_1)}{\ln (r_2/r_1)} \qquad (32)$$

r_1 = inner radius, cm.

r_2 = outer radius, cm.

l = length of tubing, cm.

Thus, the permeability is not only a function of the volume rate of diffusing gas, but also of the partial pressure of the gas, the average cross section for diffusion, and the nature and the thickness of the solid. Diffusivities in solids may also vary with temperature and with concentration. Because information on the permeability of hydrogen and methane in Tygon tubing was unavailable, it was necessary to determine these data experimentally.

Determination of Permeability

Permeabilities were determined only for hydrogen and methane because the diffusional losses of these gases were required in the COD balance calculations. The same apparatus shown in Figures 6 and 8 was used for the determination. The influent and effluent gas lines of both Systems 1 and 2 consisted of 10 foot lengths of Tygon tubing (Formula R-3603, 0.318 cm ID by 0.159 cm wall thickness). The average cross section for radial diffusion (A) of each tubing length equalled about 570 sq cm.

Gas mixtures containing hydrogen-nitrogen (50:50) or methane-nitrogen (50:50) were pumped through the reactor systems and recovered in the effluent gas reservoir. The diffusion loss of each gas was assumed to equal the volume difference between the pumped and the recovered gases. Identical temperature conditions were maintained for the permeability measurements as for the kinetic study.

The permeability was calculated from the diffusion loss of hydrogen or methane. The pressure gradient was assumed to equal an average of the partial pressure of hydrogen or methane in the feed and effluent gas reservoirs. The flow rates of the gas mixtures were selected to limit the maximum changes in partial pressure betwenn reservoirs to less than 15 percent.

The following permeabilities were calculated from an average of three determinations:

$$(Pe)_{H_2} = 6.17 \times 10^{-5} \text{ liters/day per sq. cm per atm/cm thickness} \quad (33)$$

$$(Pe)_{CH_4} = 1.89 \times 10^{-5} \text{ liters/day per sq. cm per atm/cm thickness} \quad (34)$$

For a 10 foot length of tubing of cross section (A) equal to 570 sq cm, with walls of 0.159 cm thickness, the steady state volume flow rate of hydrogen was estimated as follows:

$$(V_D)_{H_2} = 0.221 \text{ liters/day - atm hydrogen} \quad (16)$$

The similar expression for methane is as follows:

$$(V_D)_{CH_4} = 0.0676 \text{ liters/day - atm methane} \quad (17)$$

These expressions were used to compute the gas flow rates (adjusted for diffusion losses) reported in Tables XXVII and XXVIII of Appendix E.

APPENDIX E

HYDROGEN ENRICHMENT GROWTH AND KINETIC STUDY DATA

TABLE XXIV

GENERAL DATA - ENRICHMENT STUDY

Balance Period	F_o[a] ml/day	F_1[b] ml/day	F_s[c] ml/day	θ[d] days	θ_c[e] days	$(S_o)_L$[f] mg/ℓ	$(S_1)_L$[g] mg/ℓ	X_1[h] mg VSS/ℓ	pH	Volatile Acids mg HAc/ℓ	Reactor NH_3-N mg N/ℓ
						System 1					
1	103	93	6	7.26	7.61	1297	1222	122	6.93		58
2	121	99	8	6.21	7.02	1261	1326	118	6.93		
3	97	89	6	7.77	7.87	1261	1418	119	6.84		
4	95	92	8	7.87	7.50	1627	1572	125	6.73	420	
5	99	92	6	7.54	7.63	1627	1618	128	6.68		117
6	157	148	8	4.76	4.81	1627	1565	113	6.73	355	
7	163	158	6	4.60	4.58	1282	1148	104	6.90		132
8	161	149	10	4.66	4.72	1408	1141	100	6.86	210	
9	140	132	5	5.35	5.47	1451	1174	102	6.88		106
10	164	149	12	4.56	4.67	1451	1234	113	6.89	175	
11	151	148	6	4.97	4.88	1451	1228	121	7.07		125
12	174	167	8	4.31	4.30	1444	1199	136	6.92	235	
13	273	265	6	2.75	2.77	1440	1153	135	6.85		109
14	303	298	8	2.48	2.45	1440	1087	132	6.70	85	
15	297	295	6	2.53	2.50	1432	1073	139	7.32		95
16	286	286	8	2.63	2.55	1418	1091	143	7.50	200	
17	283	279	4	2.65	2.64	1375	1103	153	7.27		111
18	272	258	12	2.76	2.78	1241	1079	166	7.37		
19	291	282	6	2.58	2.60	1296	1051	166	7.30		124
						System 2					
1	31	21	8	24.41	26.21	1574	1467	140	6.96		
2	30	28	6	25.29	26.89	1334	[illegible]	150	6.96		
3	29	18	8	25.68	28.11	1284	1207	179	7.01		
4	31	22	10	24.51	23.64	1407	1137	180	6.86		
5	46	33	8	16.17	18.08	1448	1261	177	6.84		56
6	47	39	6	16.04	16.65	1344	1174	175	6.92		53
7	46	28	8	16.19	20.83	1311	1141	157	6.84		
8	37	29	6	20.55	21.82	1385	1226	163	6.78		
9	41	33	8	18.27	18.45	1385	1303	173	6.80		
10	45	36	6	16.66	17.88	1385	1395	169	6.86		111
11	41	30	8	18.36	19.74	1360	1472	156	6.86	255	
12	37	32	6	20.13	19.55	1360	1175	151	6.73		65
13	56	50	8	13.34	12.83	1358	1174	150	6.82	155	
14	64	54	5	11.75	12.71	1353	1158	134	6.97		79
15	55	36	12	13.69	15.76	1349	1141	129	7.04	95	
16	68	71	6	10.95	9.73	1344	1167	137	6.81		94
17	59	49	8	12.64	13.14	1342	1190	147	6.94	175	
18	67	57	6	11.21	11.83	1342	1158	155	6.95		96
19	50	39	6	15.15	16.79	1329	1128	157	7.08	55	
20	193	185	6	3.89	3.93	1329	1130	147	6.68		106
21	237	232	8	3.17	3.13	1470	1139	140	6.72	115	
22	238	233	4	3.15	3.16	1498	1139	150	6.72		112
23	235	218	12	3.19	3.26	1425	1137	161	6.80		
24	223	217	6	3.36	3.36	1316	1121	165	6.72		111

[a] F_o = liqui. influent rate.

[b] F_1 = reactor effluent rate.

[c] F_s = reactor sample rate.

[d] θ = hydraulic residence time, days.

[e] θ_c = cell residence time, days.

[f] $(S_o)_L$ = liquid influent soluble COD.

[g] $(S_1)_L$ = reactor and effluent soluble COD.

[h] X_1 = reactor sample VSS.

TABLE XXV

SYSTEM 1 GAS FLOW RATES AND ANALYSES

Balance Period	Influent Gas			Reactor Sample Gas				Effluent Gas			
	Flow Rate ℓ/day	H_2 %	CO_2 %	Flow Rate ℓ/day	H_2 %	CO_2 %	CH_4 %	Flow Rate ℓ/day	H_2 %	CO_2 %	CH_4 %
1	1.238	48.79	24.14	0.008	13.60	11.50	15.90	0.438	11.65	5.33	15.06
2	1.237	48.79	24.14	0.006	14.96	10.01	14.93	0.438	11.83	6.25	15.97
3	1.255	48.79	24.14	0.008	9.23	8.45	16.83	0.586	14.74	5.71	14.65
4	1.170	48.38	22.91	0.006	16.39	11.27	14.51	0.459	11.64	5.08	15.49
5	1.639	47.58	20.02	0.008	18.49	10.04	13.77	0.710	15.79	7.91	14.71
6	1.65[illegible]	47.58	20.02	0.006	15.45	7.63	14.27	0.799	17.82	7.39	14.11
7	1.638	48.66	15.20	0.008	17.65	5.51	13.68	0.817	17.65	5.51	13.68
8	1.512	48.15	22.88	0.008	16.76	8.35	15.92	0.672	15.56	4.53	13.11
9	1.119	56.50	20.57	0.005	18.39	7.23	19.74	0.412	14.66	4.35	18.75
10	1.312	56.50	20.57	0.012	20.18	6.39	19.41	0.530	18.32	3.61	18.28
11	1.306	56.50	20.57	0.006	14.94	3.88	21.70	0.605	16.15	2.75	19.50
12	1.504	58.18	19.48	0.008	21.50	6.69	20.25	0.512	18.96	2.89	18.99
13	2.457	58.97	18.98	0.006	35.02	6.58	15.46	1.283	29.70	6.63	16.78
14	2.585	65.03	23.17	0.008	38.53	4.98	15.14	1.086	38.53	4.98	15.14
15	2.202	80.52	17.71	0.006	55.83	6.34	32.40	0.864	57.24	4.51	25.52
16	3.168	81.47	16.46	0.008	57.44	5.31	31.08	0.974	54.35	4.32	32.62
17	2.810	78.26	20.83	0.004	56.06	4.12	34.03	0.943	52.65	3.67	34.15
18	2.811	79.30	20.10	0.012	51.81	2.54	37.45	0.821	47.69	2.62	38.35
19	2.952	80.04	19.50	0.006	52.26	2.89	39.22	0.927	51.71	2.86	37.30

NOTE: All flow rates expressed as volume of gas per day at 0°C and 760 mm Hg.

TABLE XXVI

SYSTEM 2 GAS FLOW RATES AND ANALYSES

Balance Period	Influent Gas			Reactor Sample Gas				Effluent Gas			
	Flow Rate ℓ/day	H_2 %	CO_2 %	Flow Rate ℓ/day	H_2 %	CO_2 %	CH_4 %	Flow Rate ℓ/day	H_2 %	CO_2 %	CH_4 %
1	1.063	17.63	23.28	0.008	2.29	9.58	3.86	0.680	2.62	6.17	3.51
2	0.971	17.63	23.28	0.006	2.02	8.28	3.62	0.707	2.45	6.10	3.48
3	0.993	17.63	23.28	0.008	2.09	7.48	3.76	0.685	2.18	5.22	3.62
4	0.960	17.69	20.78	0.006	1.85	9.14	3.43	0.581	2.09	5.31	3.51
5	1.160	17.69	20.78	0.008	1.96	10.31	4.02	0.461	2.21	6.20	3.91
6	1.469	17.69	20.78	0.006	3.97	5.13	3.42	1.034	3.97	5.13	3.42
7	1.332	21.02	23.05	0.008	6.90	10.97	4.22	0.858	3.16	5.87	3.54
8	1.172	26.92	27.12	0.006	4.00	13.11	6.61	0.647	4.51	6.59	5.80
9	1.164	26.92	27.12	0.008	4.01	12.57	6.88	0.613	3.89	6.32	7.42
10	1.204	26.92	27.12	0.006	3.89	10.54	6.48	0.717	3.83	6.15	6.47
11	1.189	27.08	25.40	0.008	4.46	10.94	6.43	0.732	3.98	4.92	6.07
12	1.201	27.53	21.61	0.006	3.50	11.56	7.04	0.633	4.40	6.16	6.70
13	1.361	39.24	17.27	0.008	7.72	10.03	10.78	0.727	6.68	6.19	8.42
14	0.934	40.63	16.64	0.005	5.12	5.27	10.53	0.455	4.83	6.72	11.83
15	0.903	40.63	16.64	0.012	5.04	6.27	11.52	0.470	4.85	4.53	10.64
16	0.930	40.63	16.64	0.006	5.62	5.18	11.36	0.482	5.62	5.18	11.36
17	0.978	41.51	16.87	0.008	5.96	5.31	10.64	0.614	5.40	3.89	9.85
18	1.056	42.08	16.87	0.006	5.68	5.25	12.45	0.457	5.17	4.20	10.56
19	1.146	42.08	16.87	0.008	7.63	3.96	11.76	0.604	7.63	3.96	11.76
20	2.022	58.65	22.01	0.006	32.20	13.90	30.37	1.537	22.84	8.20	8.91
21	2.248	72.39	26.24	0.008	29.38	12.63	40.88	0.463	31.37	5.43	39.87
22	1.962	71.36	27.32	0.004	26.21	10.81	50.99	0.388	26.65	5.11	54.96
23	1.967	71.12	27.57	0.008	29.46	7.13	51.65	0.418	24.90	3.93	56.08
24	2.079	74.36	23.52	0.006	25.15	11.22	52.61	0.395	24.39	4.59	55.41

NOTE: All flow rates expressed as volume of gas per day at 0°C and 760 mm Hg.

TABLE XXVII

SYSTEM 1 ADJUSTED GAS FLOW RATES

Balance Period	Influent H_2		Effluent H_2		Sample H_2 ℓ/day	H_2 Removed ℓ/day	Effluent CH_4		Sample CH_4 ℓ/day	CH_4 Produced ℓ/day
	Flow Rate ℓ/day	Diffusion ℓ/day	Flow Rate ℓ/day	Diffusion ℓ/day			Flow Rate ℓ/day	Diffusion ℓ/day		
1	0.604	0.116	0.051	0.028	0.001	0.464	0.066	0.010	0.001	0.077
2	0.604	0.116	0.052	0.028	0.001	0.463	0.070	0.010	0.001	0.081
3	0.612	0.116	0.086	0.035	0.001	0.444	0.086	0.010	0.001	0.097
4	0.566	0.115	0.053	0.028	0.001	0.425	0.071	0.010	0.001	0.082
5	0.780	0.113	0.112	0.038	0.001	0.592	0.104	0.010	0.001	0.115
6	0.786	0.113	0.142	0.042	0.001	0.572	0.113	0.010	0.000	0.123
7	0.797	0.116	0.144	0.042	0.001	0.578	0.112	0.009	0.001	0.122
8	0.728	0.115	0.105	0.037	0.001	0.544	0.088	0.010	0.001	0.099
9	0.632	0.134	0.060	0.035	0.001	0.472	0.077	0.013	0.000	0.090
10	0.741	0.134	0.097	0.044	0.002	0.552	0.097	0.013	0.001	0.111
11	0.738	0.134	0.098	0.038	0.001	0.543	0.118	0.014	0.000	0.132
12	0.875	0.138	0.097	0.045	0.002	0.583	0.097	0.013	0.001	0.111
13	1.449	0.140	0.381	0.071	0.002	0.997	0.215	0.011	0.000	0.226
14	1.681	0.155	0.418	0.092	0.003	1.197	0.164	0.010	0.000	0.174
15	1.773	0.191	0.495	0.136	0.003	1.220	0.221	0.020	0.000	0.242
16	2.581	0.194	0.529	0.129	0.005	1.982	0.318	0.022	0.000	0.340
17	2.199	0.186	0.497	0.125	0.002	1.639	0.322	0.023	0.000	0.345
18	2.229	0.189	0.392	0.114	0.006	1.756	0.317	0.026	0.000	0.343
19	2.363	0.191	0.479	0.123	0.003	1.813	0.346	0.026	0.000	0.372

NOTE: All flow rates expressed as volume per day at 0°C and 760 mm Hg.

TABLE XXVIII

SYSTEM 2 ADJUSTED GAS FLOW RATES

Balance Period	Influent H_2		Effluent H_2		Sample H_2 ℓ/day	H_2 Removed ℓ/day	Effluent CH_4		Sample CH_4 ℓ/day	CH_4 Produced ℓ/day
	Flow Rate ℓ/day	Diffusion ℓ/day	Flow Rate ℓ/day	Diffusion ℓ/day			Flow Rate ℓ/day	Diffusion ℓ/day		
1	0.187	0.042	0.018	0.006	0.000	0.133	0.024	0.003	0.000	0.027
2	0.171	0.042	0.017	0.006	0.000	0.118	0.025	0.002	0.000	0.027
3	0.175	0.042	0.015	0.005	0.000	0.123	0.025	0.003	0.000	0.028
4	0.170	0.042	0.012	0.005	0.000	0.121	0.020	0.002	0.000	0.022
5	0.205	0.042	0.010	0.005	0.000	0.158	0.018	0.003	0.000	0.021
6	0.260	0.042	0.041	0.009	0.000	0.188	0.035	0.002	0.000	0.037
7	0.280	0.050	0.027	0.008	0.001	0.210	0.030	0.003	0.000	0.033
8	0.316	0.064	0.029	0.011	0.000	0.234	0.038	0.004	0.000	0.042
9	0.313	0.064	0.024	0.009	0.000	0.234	0.046	0.005	0.001	0.052
10	0.324	0.064	0.028	0.009	0.000	0.241	0.046	0.004	0.000	0.050
11	0.322	0.065	0.029	0.009	0.000	0.237	0.044	0.004	0.001	0.049
12	0.331	0.066	0.028	0.010	0.000	0.237	0.042	0.005	0.000	0.047
13	0.534	0.093	0.049	0.016	0.001	0.407	0.061	0.007	0.001	0.069
14	0.380	0.097	0.022	0.012	0.000	0.273	0.054	0.008	0.001	0.063
15	0.367	0.097	0.023	0.012	0.001	0.258	0.050	0.008	0.001	0.059
16	0.378	0.097	0.027	0.013	0.000	0.267	0.055	0.008	0.001	0.064
17	0.406	0.099	0.033	0.013	0.001	0.286	0.061	0.007	0.001	0.069
18	0.444	0.100	0.024	0.012	0.000	0.332	0.048	0.008	0.001	0.057
19	0.482	0.100	0.046	0.018	0.001	0.353	0.071	0.008	0.001	0.080
20	1.186	0.140	0.351	0.054	0.002	0.747	0.137	0.013	0.002	0.152
21	1.627	0.172	0.145	0.075	0.002	1.383	0.185	0.029	0.004	0.218
22	1.400	0.170	0.103	0.063	0.001	1.189	0.213	0.036	0.002	0.251
23	1.399	0.169	0.104	0.059	0.002	1.183	0.234	0.036	0.004	0.274
24	1.546	0.177	0.096	0.058	0.002	1.329	0.219	0.036	0.003	0.258

NOTE: All flow rates expressed as volume of gas per day at 0°C and 760 mm Hg.

TABLE XXIX
COD BALANCE – ENRICHMENT STUDY

Balance Period	Influent		Effluent				Recovery %	Acetic Acid COD	
	H_2-COD mg/day	Soluble COD mg/day[a]	H_2-COD mg/day	Soluble COD mg/day[b]	CH_4-COD Produced mg/day	Cell-COD Produced mg/day[c]		mg/day[d]	Per cent of Effluent Soluble COD
				System 1					
1	348.4	133.5	57.1	120.3	220.0	17.1	86.0		
2	348.4	153.2	57.8	141.7	231.4	17.9	89.5		
3	354.1	121.7	87.1	135.1	277.1	16.1	108.3		
4	322.0	155.1	57.8	157.2	234.3	17.7	97.9	45	28.5
5	476.2	161.9	107.8	159.1	328.6	17.9	96.1		
6	480.5	256.2	132.1	243.8	351.4	25.0	102.1	59	24.2
7	486.2	209.0	133.5	188.2	348.6	24.2	99.9		
8	437.7	226.4	102.1	181.3	282.8	22.6	88.7	36	19.6
9	355.6	203.5	68.5	160.8	257.1	19.8	90.5		
10	433.4	238.7	102.1	198.1	317.1	25.8	95.7	30	15.1
11	431.3	218.9	97.8	188.8	377.1	26.4	106.1		
12	526.2	251.3	102.8	209.4	317.1	33.7	85.3	44	20.9
13	934.6	393.0	324.2	312.2	645.7	51.8	100.5		
14	1,089.6	435.8	366.3	333.0	497.1	56.7	82.1	28	8.3
15	1,129.5	425.5	452.7	322.5	691.4	59.4	98.1		
16	1,704.3	405.1	473.4	321.2	971.4	59.8	86.6	63	19.5
17	1,437.3	389.5	445.5	312.9	985.7	61.6	98.8		
18	1,456.6	337.0	365.6	291.5	980.0	63.7	94.8		
19	1,550.8	377.1	432.0	303.0	1,062.8	67.9	96.8		
				System 2					
1	103.5	48.4	17.1	41.9	77.1	5.7	93.4		
2	92.1	39.6	16.4	48.7	77.1	5.9	112.6		
3	95.0	37.5	14.3	32.2	80.0	6.7	100.5		
4	91.4	43.1	12.1	36.1	62.9	8.0	88.6		
5	116.4	67.2	10.7	52.3	60.0	10.3	72.6		
6	155.7	62.8	35.7	52.9	105.7	11.0	94.0		
7	164.2	60.7	25.7	41.1	94.3	7.9	75.1		
8	179.9	50.6	28.6	42.1	120.0	7.8	86.1		
9	177.8	56.9	23.6	53.0	148.6	10.1	100.3		
10	185.6	62.3	26.4	58.5	142.9	9.9	95.9		
11	183.5	55.6	27.1	56.3	140.0	8.3	96.9	10	18.4
12	189.2	50.7	27.1	45.1	134.3	8.1	89.5		
13	314.9	76.3	47.1	68.7	197.1	12.4	83.2	10	14.1
14	202.1	86.4	24.3	68.4	180.0	11.2	98.4		
15	192.8	73.9	25.7	54.3	168.6	8.7	96.5	5	8.9
16	200.6	92.0	28.6	89.9	182.8	15.0	108.1		
17	219.2	79.6	33.6	67.9	197.1	11.9	103.9	11	15.7
18	245.6	89.8	25.7	73.4	162.8	13.9	82.2		
19	272.7	65.8	46.4	50.4	228.6	9.9	99.1	3	5.2
20	746.8	256.1	290.6	215.7	434.3	39.8	97.8		
21	1,038.9	350.6	158.5	273.2	622.8	47.7	79.3	29	10.8
22	878.2	357.2	119.2	270.3	717.1	50.5	93.7		
23	878.2	335.3	117.8	254.3	782.8	52.5	99.5		
24	977.5	293.6	111.4	255.5	737.1	52.2	91.0		

[a] = $F_o(S_o)_L(10^{-3})$.

[b] = $(F_1 + F_S)(S_1)_L(10^{-3})$.

[c] = $1.42\ (F_1 + F_S)X_1(10^{-3})$.

[d] = $(10^{-3})(1.07)(F_1 + F_S)$(Volatile acids, mg HAc/$\ell$) .

APPENDIX F

HYDROGEN SUPPLEMENTATION STUDY DATA

TABLE XXX

SYSTEM 3 GENERAL DATA

Balance Period	F_o [a] mℓ/day	F_1 [b] mℓ/day	F_s [c] mℓ/day	θ [d] days	θ_c [e] days	$(S_o)_L$ [f] mg/ℓ	$(S_1)_L$ [g] mg/ℓ	X_1 [h] mg VSS/ℓ	X_2 [i] mg VSS/ℓ
1	199	200	11	23.8	-	8,060	1,500	320	410
2	164	175	6	27.7	-	7,800	2,040	380	530
3	199	173	6	27.9	34.9	8,070	2,180	400	320
4	187	204	6	23.9	23.5	7,760	2,040	430	440
5	196	170	6	28.5	44.4	7,760	2,000	510	330
6	192	152	6	26.1	32.4	7,950	1,720	640	630
7	192	199	6	26.0	30.8	7,840	1,580	700	550
8	210	190	6	23.9	33.8	9,630	2,030	690	510
9	193	189	6	25.9	39.1	10,850	2,610	680	440
10	192	188	6	26.0	43.3	8,940	2,980	790	460
11	197	189	10	25.4	46.3	9,560	3,200	920	480
12	198	189	6	25.2	39.7	9,420	3,440	960	610
13	165	153	6	30.2	74.0	9,620	3,750	970	390
14	164	159	6	30.4	70.7	10,080	3,840	1,040	430
15	164	165	6	30.5	67.0	10,080	3,650	1,120	470
16	176	158	6	28.5	58.8	9,800	3,320	1,160	590
17	173	164	6	28.9	44.6	9,950	3,010	1,150	750
18	168	159	6	29.7	49.4	9,980	2,830	1,150	690
19	168	158	6	29.8	46.4	10,020	2,770	1,150	740
20	162	204	6	30.9	38.0	10,150	2,750	1,140	710
21	163	102	6	30.6	94.0	10,200	2,720	1,180	550
22	170	187	6	29.4	45.6	9,860	2,620	1,160	640
23	167	135	6	29.9	58.3	9,820	2,310	1,140	680
24	180	166	6	27.7	45.3	9,600	1,950	1,170	620
25	175	168	6	28.5	46.0	9,900	1,720	1,170	720
26	173	156	6	29.0	50.0	9,920	1,610	1,170	710
27	159	164	6	31.4	45.2	9,430	1,690	1,100	700

[a] F_o = liquid influent rate.

[b] F_1 = reactor effluent rate.

[c] F_s = reactor sample rate.

[d] θ = hydraulic residence time, days.

[e] θ_c = cell residence time, days.

[f] $(S_o)_L$ = liquid influent COD.

[g] $(S_1)_L$ = reactor and effluent COD.

[h] X_1 = reactor sample VSS.

[i] X_2 = reactor effluent VSS.

TABLE XXX

SYSTEM 3 GENERAL DATA (Continued)

Balance Period	pH	Volatile Acids mg/ℓ as Acetic Acid			Alkalinity mg/ℓ as $CaCO_3$	Effluent[a] Gas ℓ/day	CH_4 %	H_2 %	CO_2 %	mg Acetic Acid / mg VSS
		Total	Acetic	Propionic						
1	6.44	-	-	-	690	0.125	16.3	-	3.2	-
2	6.83	1,370	710	410	1,100	0.352	66.7	-	11.7	1.86
3	6.93	745	65	465	1,380	0.374	74.2	-	9.2	0.16
4	7.23	645	95	455	1,350	0.404	73.8	-	13.6	0.22
5	7.10	520	70	365	1,610	0.388	69.3	-	14.5	0.14
6	7.13	255	60	145	1,690	0.588	73.8	-	14.2	0.09
7	7.34	235	120	60	1,700	0.504	67.5	6.5	9.1	0.17
8	7.30	780	480	140	1,650	0.520	67.4	10.2	11.0	0.70
9	7.22	590	300	130	1,610	0.461	70.4	6.5	9.8	0.44
10	7.10	1,260	450	480	1,570	0.478	71.9	4.9	8.9	0.57
11	7.10	1,140	620	360	1,620	0.482	72.4	7.9	7.6	0.68
12	7.01	1,365	830	310	1,610	0.473	74.0	6.7	10.0	0.87
13	6.91	1,665	1,030	400	1,820	0.302	76.0	0.7	6.6	1.06
14	7.00	1,435	790	395	1,940	0.359	73.3	-	10.6	0.76
15	6.97	1,260	480	515	2,050	0.448	73.5	-	13.3	0.43
16	7.07	1,210	440	620	2,140	0.533	76.3	-	14.6	0.38
17	7.10	995	310	630	2,190	0.491	75.8	-	15.7	0.27
18	7.13	1,195	310	800	2,210	0.445	76.9	-	13.2	0.27
19	7.33	1,090	170	685	2,380	0.437	75.5	-	14.0	0.15
20	7.16	1,010	190	660	2,000	0.417	72.9	-	14.2	0.18
21	7.11	1,000	155	730	2,000	0.497	73.5	-	14.9	0.13
22	7.00	820	160	490	2,070	0.475	75.7	-	13.3	0.14
23	7.21	495	120	235	2,420	0.581	75.7	-	15.7	0.10
24	7.23	400	90	100	2,460	0.595	75.7	-	14.9	0.08
25	7.30	265	45	45	2,600	0.622	73.4	-	15.6	0.04
26	7.27	270	0	55	2,090	0.571	64.4	-	18.2	0.00
27	7.21	-	-	-	2,100	0.499	61.4	-	15.5	-

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[a] Expressed at 0°C and 760 mm Hg.

TABLE XXXI

SYSTEM 4 GENERAL DATA

Balance Period	F_O [a] mℓ/day	F_1 [b] mℓ/day	F_S [c] mℓ/day	θ [d] days	θ_c [e] days	$(S_o)_L$ [f] mg/ℓ	$(S_1)_L$ [g] mg/ℓ	X_1 [h] mg VSS/ℓ	X_2 [i] mg VSS/ℓ
1	196	168	11	28.0	-	8,000	1,530	300	510
2	171	144	6	33.5	-	7,790	2,100	350	580
3	205	191	6	25.5	30.9	8,070	2,240	470	380
4	193	167	6	29.0	39.6	7,760	2,070	500	360
5	199	209	6	25.1	30.1	7,760	2,020	570	440
6	197	228	6	25.4	36.4	7,950	1,780	750	500
7	197	192	6	25.4	34.7	7,840	1,400	800	530
8	198	192	6	25.2	46.8	9,630	1,300	830	400
9	199	192	6	25.2	43.3	10,850	1,330	920	500
10	207	200	6	24.1	37.8	8,940	1,420	1,030	590
11	206	195	10	24.3	35.7	9,560	1,580	1,070	690
12	202	181	6	24.7	45.6	9,420	1,670	1,080	620
13	197	190	6	25.4	42.0	9,620	1,840	1,090	650
14	204	196	6	24.5	63.6	10,080	2,070	1,170	430
15	201	191	6	24.8	63.2	10,080	2,270	1,290	490
16	208	199	6	24.0	63.6	9,800	2,480	1,390	510
17	214	203	6	23.4	67.8	9,950	2,710	1,530	510
18	205	194	6	24.4	62.8	9,980	2,820	1,580	600
19	204	194	6	24.5	62.3	10,020	3,040	1,580	610
20	197	188	6	25.4	61.0	10,150	3,440	1,520	620
21	186	190	6	27.0	61.3	10,200	3,750	1,480	590
22	205	197	6	24.4	59.2	9,860	3,910	1,400	560
23	198	191	6	25.2	49.4	9,820	4,000	1,280	630
24	209	199	6	24.0	46.1	9,600	4,070	1,280	660
25	206	198	6	24.3	52.6	9,900	4,120	1,290	580
26	196	185	6	25.7	51.3	9,920	4,240	1,280	640
27	189	180	6	26.4	52.0	9,430	4,370	1,260	630

[a] F_O = liquid influent rate.

[b] F_1 = reactor effluent rate.

[c] F_S = reactor sample rate.

[d] θ = hydraulic residence time, days.

[e] θ_c = cell residence time, days.

[f] $(S_o)_L$ = liquid influent COD.

[g] $(S_1)_L$ = reactor and effluent COD.

[h] X_1 = reactor sample VSS.

[i] X_2 = reactor effluent VSS.

TABLE XXXI

SYSTEM 4 GENERAL DATA (Continued)

Balance Period	pH	Volatile Acids mg/ℓ as Acetic Acid			Alkalinity mg/ℓ as $CaCO_3$	Effluent[a] Gas ℓ/day	CH_4 %	H_2 %	CO_2 %	mg Acetic Acid / mg VSS
		Total	Acetic	Propionic						
1	6.43	-	-	-	740	0.143	23.3	-	6.1	-
2	6.90	1,325	745	360	1,040	0.345	71.7	-	14.7	2.11
3	6.84	655	55	450	1,360	0.338	76.4	-	10.5	0.12
4	7.24	610	115	425	1,400	0.458	74.7	-	15.8	0.23
5	7.09	485	65	345	1,600	0.433	74.1	-	15.9	0.11
6	7.12	335	30	245	1,700	0.508	77.2	-	14.4	0.04
7	7.28	130	60	15	1,780	0.536	74.6	-	14.4	0.07
8	7.27	235	140	35	1,940	0.530	78.7	-	15.8	0.17
9	7.19	190	135	25	1,940	0.708	75.8	-	17.8	0.15
10	7.14	355	250	55	1,900	0.607	77.0	-	15.5	0.24
11	7.16	290	185	55	2,100	0.538	75.8	-	16.8	0.17
12	7.08	365	220	90	2,110	0.581	72.5	-	17.0	0.20
13	7.09	540	340	145	2,160	0.521	75.5	-	15.1	0.31
14	7.23	660	380	195	2,160	0.503	76.4	-	15.5	0.33
15	7.06	690	320	245	2,170	0.530	73.2	-	16.7	0.25
16	7.10	745	330	340	2,210	0.540	70.8	-	17.0	0.24
17	7.08	920	360	465	2,210	0.506	75.6	-	15.9	0.23
18	7.08	1,060	300	690	2,250	0.500	75.8	-	16.5	0.19
19	7.22	1,360	440	720	2,160	0.567	68.0	2.9	15.3	0.28
20	7.19	1,540	550	735	2,070	0.511	66.3	7.2	11.5	0.36
21	7.21	1,240	220	720	2,120	0.540	67.5	12.3	10.3	0.15
22	7.04	1,635	485	755	2,000	0.601	70.0	8.4	7.8	0.35
23	7.22	1,520	380	750	2,120	0.536	68.7	9.3	9.0	0.30
24	7.18	1,765	265	1,045	2,340	0.545	69.1	10.5	10.6	0.21
25	7.15	1,310	250	735	2,240	0.380	76.1	-	12.8	0.19
26	6.99	1,650	560	735	1,820	0.339	71.7	-	17.5	0.44
27	6.82	-	-	-	2,090	0.335	71.1	-	16.6	-

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[a] Expressed at 0°C and 760 mm Hg.

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